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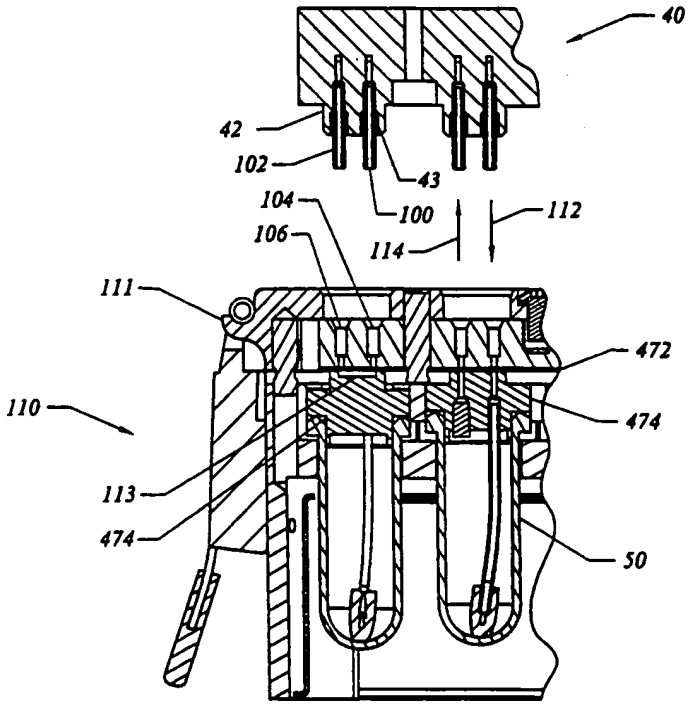
(57) Abstract

The present system is useful for the synthesis of chemical compounds and can be used for solid state or liquid chemistries. In one embodiment, the system (10) includes a reagent delivery/vent unit (20) and a chemical processing unit (30) containing a plurality of reaction vessels (50). The reagent delivery/vent unit has a plurality of tubular structures (100) adapted to slidably engage cavities (104) in the chemical processing unit to form a circumferential seal about each of the tubular structures. The delivery/vent unit typically uses a fluid interface head containing the tubular structures to engage the chemical processing unit. The chemical processing unit preferably uses a cassette (110) to house all of the reaction vessels and defines the plurality of elongate cavities adapted to receive the tubular structures.



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SYSTEMS AND METHODS FOR COMBINATORIAL ORGANIC SYNTHESIS OF ARRAYS OF REACTION

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional U.S. Patent Application Serial No. 60/097,508, filed on August 21, 1998 (Attorney Docket No. 16925-001910) and provisional U.S. Patent Application Serial No. 60/063,134 filed on October 22, 1997 (Attorney Docket No. 16925-001900). The full disclosures of each of these patent applications are incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

The standard method for searching for new chemical compounds which can effectively modulate biological processes employs the screening of pre-existing compounds in assays which have been designed to test particular properties of the compound being screened. Similarly, in designing compounds having desired physiochemical properties for general chemical applications, numerous compounds must be individually prepared and tested.

To reduce the time and expense involved in preparing and screening a large number of compounds for biological activity or for desirable physiochemical properties, technology has been developed for providing libraries of compounds for the discovery of lead compounds. Current methods for generating large numbers of molecularly diverse compounds focus on the use of solid phase synthesis. The generation of combinatorial libraries of chemical compounds by employing solid phase synthesis is well known in the art. For example, Geysen, et al. (Proc. Natl. Acad. Sci. USA, 3998 (1984) describe the construction of multi-amino acid peptide libraries; Houghton, et al. (Nature, 354, 84 (199 1) and PCT Patent Pub. No. WO 92/09300) describe the generation and use

of synthetic peptide combinatorial libraries for basic research and drug discovery; Lam, et al. (Nature, 354, 82 (199 1) and PCT Patent Pub. No. WO 92/0009 1) describe a method of synthesis of linear peptides on a solid support such as polystyrene or polyacrylamide resin.

The growing importance of combinatorial chemistry as an integral component of the drug discovery process has spurred extensive technological and synthetic advances in the field (Thompson, L. A.; Ellman, J. A. (1996) *Chem. Rev.* 96,555-600). Founded in peptide synthesis devised by Merrifield, solid phase chemistry has emerged as the preeminent method for construction of small molecule combinatorial libraries (see e.g. Merrifield, R B. (I 963) *J. Am. Chem. Soc.* 85, 21492154; (a) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *(1995)Tetrahedron* 51(30), 8135-8173. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. (1994) *J. Med Chem.* 37,1385-1401.).

Unfortunately, the generation of chemical compounds for combinatorial chemical libraries is a labor intensive process. Working with numerous reaction vessels concurrently is very difficult and time consuming. In the past, multiple solid phase reactions were conducted by heating a substrate attached to resin beads with appropriate reagents and solvents in a test tube immersed in a hot oil bath with a rotating magnetic stir bar. Draining was accomplished by pouring the contents of the test tube through a filter. Back and forth operation between reacting and draining operations was very tedious and potentially exposed the reaction mixture to air. Certain chemical processes also required that the chemical reagents be kept under an inert or anhydrous atmosphere to prevent reactive groups from reacting with molecular oxygen, water vapor, or other agents commonly found in air.

While certain chemical synthesizers are known in the art, these synthesizers fail to provide the desired features necessary to efficiently generate large numbers of chemical compounds. Furthermore, conventional systems which use septums to seal against contamination eventually fail due to

needle punctures used to inject reagents. Incompatible reagents mixing resulting in precipitate and clogging in the plumbing system prior to delivery also causes problems.

Accordingly, there is a need for a device which would provide heating and/or cooling, mixing, a closed environment for moisture sensitive and air sensitive chemistries, easy draining, and rinsing of a plurality of reaction vessels. It would further be advantageous if the system could deliver small amounts of reagents without diluting the reaction vessel with large amounts of solvents.

SUMMARY OF THE INVENTION

The present invention is directed to a system which is useful for the synthesis of chemical compounds, for example, for the preparation of multiple discrete compounds or for combinatorial libraries of compounds. The present invention can be used for developing new drugs and chemical entities. The invention is useful for rapidly generating and systematically synthesizing large numbers of molecules that may vary in their chemical structure or composition. The invention is further useful for randomly generating a large number of candidate compounds, then later optimizing those compounds which exhibit the most desirable properties. The present system is applicable to both solid state and liquid chemistries.

The present invention preferably allows a user to minimize the cost associated with the most expensive portions of a synthesis system, such as the fluid delivery system, while maximizing the number of reaction vessels that can be serviced by the fluid delivery system. Since the delivery system typically does not need to be attached to the reaction vessels while the reactions are processing, the delivery system can make its delivery of reagents to one or more reaction vessels and then move to service other reaction vessels. The present invention can form reliable air/liquid tight seals between the delivery system and the reaction vessel without the risk of reduced seal integrity after repeated use associated with conventional septum systems.

Advantageously, the fluid pathways of the present delivery system can also be flushed prior to fluid delivery to remove contaminants which may have entered the system. This flush typically occurs after the delivery system is engaged to the reaction vessels and eliminates or significantly reduces the risk of contamination. The present invention further allows delivery of small quantities of reagents without significant dilution as often required in conventional systems. This is particularly useful in situations where the reagents are expensive or excessive amounts of solvents may interfere with the synthesis in the reaction vessel. The present invention also allows reaction vessels to be maintained at positive gas pressures, something that may not be possible in septum-based systems which tend to leak when pressurized.

In a first aspect, the present invention provides a system for the synthesis of combinatorial chemical libraries. The system includes a reagent delivery/vent unit and a chemical processing unit containing a plurality of reaction vessels. The reagent delivery/vent unit has a plurality of tubular structures adapted to slidably engage recesses or cavities in the chemical processing unit to form a circumferential seal about at least one of the tubular structures. These circumferential seals are much less likely to fail than conventional septum systems which use needles that leave behind punctures in the septum material. The delivery/vent unit typically uses a fluid interface head containing the tubular structures to engage the chemical processing unit. The chemical processing unit preferably uses a cassette to house the reaction vessels and the cassette defines the plurality of cavities adapted to receive the tubular structures. In an alternative embodiment, the tubular structures may be located on the chemical processing unit and the recesses located on the delivery/vent unit. Using the cassette advantageously allows the reaction vessels to be fluidly coupled to the interface head for reagent delivery and then be disconnected for possible offsite processing.

In a second aspect, the present invention provides a system for delivering reagents into a reaction vessel where

dilution of the reagent during delivery is preferably minimized. The system contains an interface head fluidly couplable to the reaction vessel. The interface head is preferably detachable or removable to be fluidly coupled with other reaction vessels while a first set of vessels are processing. The interface head defines a fluid pathway to the reaction vessel when it is fluidly coupled to the reaction vessel. The system uses a holding reservoir fluidly coupled with the interface head to help deliver reagents or solvents. In some embodiments, the holding reservoir may also be defined by the interface head. A valve located at an inlet of the holding reservoir controls flow into the reservoir. A pressurized gas source fluidly coupled to the valve is used to flow fluid into the reaction vessel.

The system preferably includes a syringe pump adapted to deliver a fluid bolus that includes a reagent portion and a solvent portion. The fluid bolus generally includes a small gas gap between the reagent portion and the solvent portion. This gas gap substantially minimizes dilution between reagent and solvent, thus reducing the amount of reagent used in delivery. At delivery, the fluid bolus preferably extends from the syringe pump through the delivery tubing to the holding reservoir. The amount of fluid to be delivered to the reaction vessel is pushed by the syringe pump via the relatively incompressible fluid column or bolus into the holding reservoir. The use of an isolatable, holding tube from which the fluid or reagent can be delivered to a reaction vessel allows for fluid delivery without introduction of solvent rinse fluids and this minimizes dilution. Preferably, by isolating the desired portion in the holding reservoir, the liquid column used to accurately meter the reagent into the holding reservoir can be discarded or otherwise processed while the reagent is injected a short distance to the reaction vessel. The holding reservoir fluid is preferably delivered in the last short distance into the reaction vessel via pressurized gas. Conventional systems typically do not have such metering capability so close to the reaction vessel. Conventional systems typically meter the reagent at distances

far from the reaction vessel. By the time the reagent bolus reaches the reaction vessel, it has lost much of its content in the form of droplets along the delivery tubing. Hence, without using a solvent rinse fluid to "pickup" the droplets left behind, conventional systems have difficulty ensuring that the reagent amount metered by the pump actually reaches the reaction vessel.

Embodiments of the present invention also use gas flow sensors fluidly coupled to the pressurized gas source to detect the flow of pressurized gas delivering fluid into the reaction vessel. A method according to the present invention for testing the successful delivery of liquid in a synthesis apparatus comprise the steps of flowing liquid towards a target site, flowing gas to move the liquid, measuring the gas flow rate, emptying the liquid to the target site, and measuring an increase in gas flow rate. This allows the system to detect blockages in the delivery system when gas flow is not at the level expected. Liquid sensors using optical or other sensing capability may also be used to detect whether reagents or solvents have reached their destinations.

In a further aspect, the present invention provides an improved thermal agitation unit for use in chemical synthesis. The apparatus includes a heat exchanger, a gas recirculation unit, and a gas distribution plate adapted to evenly distribute heat-exchanged gas around the reactions vessels within a temperature variance of ±5°C. The system also includes a reciprocating agitator adapted to agitate the cassette and the reaction vessels therein. The present apparatus advantageously allows for agitation of the entire cassette. Typically, no motion-limiting items such as a magnetic agitator, heating elements, or stir mechanism are attached to each reaction vessel. This provides for a more elegant design. Additionally, gas or air based thermal units advantageously allow the use of very small reaction vessels which cannot contain conventional magnetic agitation devices. Small reaction vessels are advantageous since they do not require as much reagent, which can be expensive.

In a still further aspect, the present invention provides a method for minimizing mechanical motion and/or reagent washes used in the synthesis of combinatorial chemical libraries using a plurality of reagents. The method uses a Hilbert space-filling curve to calculate the desired placement of synthesis procedures. The method includes generating a Hilbert space by sizing upward a reagent space. A fluid delivery system is slidably engaged to a reaction vessel and reagents are delivered into the reaction vessel. The use of the Hilbert space minimize the amount of motion required by the fluid interface head. This is particularly advantageous since the amount of reagent and solvent used to flush and then prime the fluid lines can be significant if there are large number of connections and disconnections.

A further understanding of the nature and advantages of the invention will become apparent by reference to the remaining portions of the specification and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows a simplified schematic view of a system according to the present invention;

Fig. 1B illustrates an exemplary embodiment of the system according to the present invention;

Fig. 2A shows a partial cross-section view of an interface head and cassette according to the present invention;

Fig. 2B-2C illustrate alternative embodiments of a removable coupling according to the present invention;

Figs. 3A-3B depict two embodiments of the interphase head;

Fig. 4 shows a tubular structure about to engage a recess in a cassette of a system according to the present invention;

Fig. 5 is a perspective view of one embodiment of a partially disassembled cassette mounted on an agitation thermal unit according to the present invention;

Figs. 6-8 show additional views of an interface head according to the present invention;

Figs. 9-10 are schematic plumbing diagrams of the system according to the present invention;

Figs. 11-14 illustrate fluid delivery using the system of Figs. 9-10 to minimize reagent dilution;

Fig. 15A is a cross-sectional view of one embodiment of the agitation thermal unit;

Fig. 15B illustrate embodiments of the heat exchange element and gas distribution plates according to the present invention;

Fig. 16 shows an agitation thermal unit having a fan positioned to generate air or gas flow over a cassette on the unit; and

Fig. 17A-17C shows the use of gas flow meters to detect the successful delivery of fluids into a reaction vessel.

DESCRIPTION OF THE PREFERRED EMBODIMENT

I. Introduction

The present invention is directed to the synthesis of chemical compounds, such as for the generation of combinatorial chemical libraries. Specifically, the present invention provides an apparatus by which any variety of single compounds or combinatorial libraries may be created. The reaction apparatus of the present invention provides numerous advantages over known instrumentation. With large numbers of samples to process, the present apparatus facilitates the synthesis by allowing for common introduction of reagents and the simultaneous washing of a plurality of reaction vessels. This processing is preferably performed under an inert atmosphere in the reaction vessels. The present invention may also provide an agitator for uniformly and gently mixing the reaction media. Constant and evenly distributed heating and cooling may be provided during synthesis.

To facilitate the ease of operation, certain functions of the present invention, such as agitation of the

reaction mixture, heating and cooling of the reaction vessel, inlet of inert atmosphere, introduction of reagents and solvents, rinsing and draining of reaction mixtures, and the like are preferably conducted by robotic automation or computer control. Accordingly, certain embodiments of the present invention are directed to the use of the apparatus which is partially or entirely conducted by robotic automation or under computer control.

As will be readily apparent to one skilled in the art the present invention is useful for the solid phase synthesis of organic compounds, including peptides. This device may be used for both solid phase chemistry and liquid-liquid chemistry. Alternatively, the present invention may be employed for the synthesis of organic compounds in the solution phase.

For the synthesis of compounds, appropriate starting materials may be attached to a support. Preferred support materials include solid polymeric materials, such as polyacrylamide, polydextran, polyethylene glycol, polystyrene, cellulose, sephadex, resins, combinations thereof, and the like. Alternate support materials include glass, acrylic, latex, and ceramics. Synthetic reactions may be conducted on the support-bound starting materials to obtain the desired compounds which may then be cleaved from the support.

As will be readily apparent to one skilled in the art, the present invention may be employed in essentially any synthetic reaction. Thus, the present invention is useful in almost all of the synthetic reactions which are known to one of skill in the art, including, for example, peptide synthesis, acylation, alkylation, condensation, cyclization, halogenation, heterogeneous catalysis, hydrolysis, metallation, nitration, nucleophilic displacement, organometallic reactions, oxidation, reduction, sulfonation, acid chloride formation, Diels-Alder reaction, Friedel-Crafts reactions, Fischer indole synthesis, Michael reactions, and the like (see e.g., H. 0. House, "Modem Synthetic Reactions", 2nd ed. (Benjamin/Cummings, Menlo Park 1972); J. March, "Advanced Organic Chemistry", 3rd ed., (John Wiley & Sons, New

York, 1985); Fieser and Fieser, "Reagents for Organic Synthesis", Volumes lend (Wiley Interscience, New York)). Likewise, the present invention has application in essentially any synthetic reaction which may be conducted in solution or on solid phase supports, including acetal formation, alkylations, alkynation, chiral alkylation, reductive alkylation, carbanion reactions, Grignard reactions, organocadmium/manganese reactions, organolidiim reactions, organozinc reactions, carbene insertion, condensations, Claisen reactions, aldol reactions, Dieckmann cyclization, Knoevenagel condensations, mannich reactions, cycloadditions, cyclizations (in particular to form heterocyclic rings), Friedel-Crafbs reactions, halogenation, bromination, chlorination, nucleophilic addition, Michael addition, aromatic nucleophilic substitution, Finkelstein reaction, Mitsunobu reaction, palladium (0) catalyzed reactions, Stille coupling, Suzuki coupling, Heck reaction, carbamate/urea formation, oxidation of primary alcohol to aldehyde, Sharpless reaction, oxidation of secondary alcohol to ketone, oxidation of aldehyde to carboxylic acid, epoxidation, oxidation of primary chloride to aldehyde, oxidative phenol coupling, reduction of acid to alcohol, reduction of aldehyde to alcohol, reduction of alkyne to alkene, reduction of amide to amine, reduction of aryl nitro to amine, reduction of azide to amine, reduction of ester to alcohol, reduction of imine to amine, reduction of iodide to alkyl, reduction of ketone to alcohol, Wittig reaction, Homer-Emmons condensation, and the like (see generally, "Solid Phase Organic Chemistry (SPOC)" and "Solid Phase Inorganic Chemistry (SPIC)", Chiron Mimotopes, pp. 1-31 (August 1995).

A "combinatorial library" is a collection of compounds in which the compounds comprising the collection are composed of one or more subunits or monomeric units (i.e. synthons). The subunits may be selected from natural or unnatural moieties including amino acids, nucleotides, sugars, lipids, carbohydrates, dienes, dienopholes, and the like. The compounds of the combinatorial library differ in one or more

ways with respect to the type(s), number, order or modification of the subunits comprising the compounds.

Combinatorial libraries generated by the methods of the present invention may be screened for pharmacologically or diagnostically useful compounds, as well as for desired physical or chemical properties. It will be clear to one skilled in the art that such screening may be conducted on a library of compounds which have been separated from the polyvalent support, or may be conducted directly on the library of compounds which are still linked to the polyvalent support.

The term "tubular structure" as used herein includes objects of a variety of shapes and containing at least one lumen. It should be understood, however, that the structure may also have a plurality of lumens. The structure is preferably an elongate member where the lumen therein extends from a proximal end to a distal end of the member. They may have circular, square, octagonal, or other cross-sectional shapes. Preferably, the structure has a smooth outer surface along which a circumferential seal may be formed.

A. Overview of Apparatus

Referring first to Fig. 1, a system 10 for the synthesis of combinatorial chemical libraries is depicted in a simplified schematic diagram. Fig. 1 shows an overview where the elements are generally grouped as either part of a reagent delivery/vent unit 20 or as part of a chemical processing unit 30. Although no limited in such manner, the delivery/vent unit 20 may include the diversity reagent sources, the solvent sources, pumps for delivering the reagent and solvents, and/or final product collection devices. The chemical processing unit 30 may include the cassette, reaction vessels, and/or agitation/thermal processing units used to bring about synthesis once the reagents have been delivered.

In a preferred embodiment, a fluidic interface head 40 is used to removably couple the elements of the delivery/vent unit 20 to the chemical processing unit 30. As

described below in further detail, the interface head 40 uses tubular structures to slidably engage cavities or openings in chemical processing unit 30. These openings or cavities are typically elongate openings located in the cassette 110, usually in lid 111. Although not restricted in this manner, the tubular structures are preferably non-coring, non-piercing structure. They may have a variety of configurations, so long as they provide an air/liquid tight seal with the cassette. The seal is preferably formed near the distal end of the tubular structure and is most preferably a circumferential seal. This releasable coupling allows the delivery/vent unit 20 to form releasable air tight seals that do not degrade over time like conventional septum seals. The releasible quality allows high-cost equipment such as the interphase head 40 and the delivery/vent unit 20 to service a plurality of chemical processing units 30. This increases production throughput at a lower cost. The present invention, as described below, also allows for the flushing of the tubular structures of unit 20 and its fluid pathways so that the risk of contamination is reduced.

Fig. 1B shows an overview of an exemplary embodiment of the present automated synthesis system 10. The system 10, manufactured as the Trident™ Automated Library Synthesis System by Argonaut Technologies, Inc. of San Carlos, California, typically has four agitation thermal units (ATU) 468. The interface head 40 may be designed to mate with various numbers of openings on the cassette 110 of each ATU 468. The interface head 40 may also have various sensors, such as light-based sensors, to monitor and visually verify the lowering and raising of the head 40 to and from cassette 110. A computer or logic device is used to control the synthesis process.

The interface head 40 and chemical processing unit 30 are typically located in fume hoods evacuating area 41. As can be seen from the Fig. 1B, the area 44 houses both the autosampler 60 and the fraction collector (Fig. 10B). The needles for the autosampler 60 and/or the fraction collector may be installed on one sliding carriage 320 which is

typically powered by step motors (not shown) to move as desired. The interface head 40 is depicted as moving on a gantry 45 and is typically moved by an x-z axis motor, which allows motion in two degrees of freedom.

While not limited to such chemistries, preferred embodiments of the present invention are adapted for use with highly corrosive chemicals used in solid phase synthesis and solution synthesis. This often requires using only borosilicate glass or teflon for those area in direct contact with the chemistry. Preferred embodiments also fill the reaction vessels 50 with inert gas such as argon or nitrogen to prevent contaminating air or moisture sensitive chemistries. As shown, the entire system 10 may be enclosed by its own fume hood and exhaust system.

B. Fluidic Interface Head

One of the components of the present invention is the fluidic interface head that is described in co-pending, commonly assigned U.S. Patent Application No. 60/063,134 (Attorney Docket No. 16925-001900) filed on October 22, 1997, the full disclosure of which has been previously incorporated herein by reference.

Referring now to Fig. 2A, the fluidic interface head 40 will now be described in further detail. Fig. 2A shows a cross-section of one embodiment of the interface head 40 mated to a cassette 110. As depicted in Figs. 3A and 3B, the head 40 may be a single linear array 90 or a larger matrix 92. Different interface head 40 sizes typically embody different advantages. The larger matrix 92 can fill many more reaction vessels 50 simultaneously, but it also requires additional fluid conduit lines. This may be a disadvantage when very small quantities of chemicals are being sent down to the reaction vessels 50. However, for concentration-tolerant processes, this may be a nonissue. Preferred embodiments of the present invention use a fluid head similar to array 90 to reduce the amount of fluid lines and plumbing required to deliver reagents to reaction vessels 50.

As shown in Fig. 2A, the interface head 40 has two extensions or tubular structures 100 and 102 which engage recesses or cavities 104 and 106 when the interface head 40 is lowered into the top of cassette 110 (as shown more clearly in Fig. 5). The cavity 104 may have an elongate or other shape to seal with the tubular structures. When engaged with the cassette 110, the tubular structures 100 and 102 form an air/liquid-tight seal with the cavities 104 and 106. The seal is preferably a circumferential seal, but it should be understood that in alternative embodiments, the seal may be a distal, end seal if sufficient compression is supplied to push the tubular structure 100 against cavity 104. Further alternative embodiments of the releasible coupling between a cassette 110 and an interface head are shown in Figs. 2B-2C and also described in commonly assigned, copending U.S. Provisional Patent Application No. 60/097,511 (Attorney Docket No. 16925-002200) filed on August 21, 1998, the full disclosure of which is incorporated herein by reference for all purposes. The tubes/extensions and recesses may assume a variety of shapes such as cylindrical or rectilinear so long as they conform to form an air/liquid-tight seal between the interface head 40, the cassette 110, and reaction vessel 50. In some embodiments, they are blunt, non-piercing and typically non-coring members. This releasable coupling provides a closed system with the reaction vessels 50 as shown in Fig. 10 when the interface head 40 and cassette 110 are engaged.

The interference to provide the desired seal between the tubular structure 100 and the side wall of cavity 104 is about 0.003" to 0.006". Preferably this seal is a circumferential seal between the structure 100 and the side walls 103 of the cavity 104 as illustrated in Fig. 4. The tubular structure 100 may have a diameter between about 0.080-0.100", preferably about 0.090" diameter. This provides an air/liquid-tight connection and ensures an inert environment for delivery of reagents or solvent washes. As seen in Fig. 2A, the tubing or conduits leading to the reaction vessels 50 of cassette 110 may also be flushed while the head 40 is

connected to the cassette and prior to delivery of fluid. The cap valve 474 may be rotated to a position where a fluid connection 113 can be formed between tubular structures 100 and 102 when they engage the lid 111 of cassette 110. This flushing further ensures the removal of contaminants prior to introduction of reagents or solvents into the reaction vessel. Details regarding contamination flushing can be found in co-pending, commonly assigned U.S. Patent Application No. 09/095,731 (Attorney Docket No. 16925-001710) filed on June 10, 1998, the full disclosure of which is incorporated herein by reference for all purposes.

The structure 100 is preferably made of a resilient, chemically inert material such as Teflon® or specifically Fluorinated Ethylene Propylene (FEP). Other fluoropolymers such as Polytetrafluoroethylene (PTFE), Tefzel (ETFE), and PFA may also be used. This provides for a reliable seal while maintaining this fluid pathway inert to the chemistries used in chemical synthesis. Further details can be found in co-pending, commonly assigned U.S. Patent Application No. 09/095,731 previously incorporated herein by reference.

In preferred embodiments, the tubular structure 100 is a smooth extruded tube of FEP. FEP is a material which cannot be easily shaped or drilled without causing brittleness or an unsmooth surface, except in an extrusion process. The present invention uses an extruded tube which is inserted into support 42 of the interface head 40. The tube 100 may be press-fit into the support 42. In an exemplary embodiment, threading such as that provided by an internally and typically externally threaded annular body 43 is used to hold the connector to the support body 42 during coupling an decoupling with the cassette 110. The tube 100, typically made of FEP, is screwed through threads such as 3-56 threads in the annular body 43 using a pin vise. The threads 45 (Figs. 4 and 7) prevent the tube 100 from being pulled out of the interface head 40 when the head is removed from the cassette 110. Excessive temperature variation such as between 150°C and -40°C of the cassette 110, may cause the cavity 104 of the cassette 110 to tightly grip the connector 100 when the

interface head is being decoupled. It should be noted that the inert pathway is maintained since a proximal end of connector or tube 100 extends beyond the threaded annular portion 43 to connect with port 95 in the support 42 (Fig. 7).

As shown by arrow 112, chemicals entering the reaction vessel 50 typically force gas out as indicated by arrow 114. Of course, it should be understood, that this process may be reversed to force chemicals out of the reaction vessel 50 by pressurizing the reaction vessel with inert gas. In preferred embodiments, the connections for both delivery and extraction are located on one side of the cassette.

Referring to Fig. 5, an exemplary embodiment of an interface head 40 and a cassette 110 mounted on an agitation thermal unit 120 is depicted. Having a releasable coupling ability, the interface head 40 may be moved successively as indicated by arrows 122 to sequentially engage all of the reaction vessels 50 housed in the cassette 110. The lid 111 of the cassette has openings 113 for aligning with guidepins in the interface head 40 as described in co-pending, commonly assigned U.S. Provisional Patent Application No. 60/097,511 (Attorney Docket No. 16925-002200) filed on August 21, 1998, the full disclosure of which has been incorporated herein by reference for all purposes. The guidepins prevent engagement of the lid 111 and interface head 40 when they are not in alignment.

The releasable coupling ability of system 10 is particularly advantageous as one major cost of these synthesis systems lies in the fluidics, plumbing, and metering devices used to deliver chemicals to the interface head 40. Once the chemicals are delivered, the services of the interface head 40 may not be needed for several more hours while reactions take place in the reaction vessels 50. Hence, by making the interface head 40 movable, many more reaction vessels 50 may be serviced without substantially increasing cost. Additionally, since the present invention uses a releasible air-tight coupling with the cassette 110 and the reaction vessels 50, the closed atmosphere in the reaction vessels 50 can be maintained even though the interface head 40 engages

and disengages the device. Furthermore, cassettes 110 may also be removed from the system 10 for offsite processing or long term incubation while other cassettes are loaded onto the system for service from the interface head 40. Details on the cassette 110 and reaction vessels 50 may be found in commonly assigned, copending U.S. Patent Application Serial No. 09/095,731 (Attorney Docket No. 16925-001710) filed on June 10, 1998, the complete disclosure of which has been previously incorporated herein by reference.

Also shown in Fig. 5, the cassette 110 is reciprocated by a cam device 130 powered by a motor 140. This is an exemplary embodiment of an agitation device used to agitate the reaction vessels 50 in the cassette 110. The cassette 110 preferably moves in a reciprocal manner as indicated by arrow 132. Reaction vessel 50 agitation is also described in commonly assigned, copending U.S. Patent Application Serial No. 09/095,731 (Attorney Docket No. 16925-001710) filed on June 10, 1998. Agitation is also described below in the description of the agitation thermal unit (ATU). The cassette 110 is removable from the agitation thermal unit 120 by pulling on a resin handle 111, as indicated by arrow 112 (Fig. 5 and Fig. 16). The handle 111 is preferably made of a material which is substantially thermally resistant.

As shown in Fig. 6, the fluidic interface head 40 may be designed to connect with one row of openings on the lid 111 of cassette 110. The interface head 40 shown in Fig. 6 is designed for use with an automated synthesis device as shown in Fig. 1B. The interface head 40 is typically mounted on an x-z axis manipulator that can move the interface head 40 to various positions over the cassettes 110 (Fig. 1B) and lower the head onto the cassettes. The openings 92 are typically occupied by an interface tube 100. Additional details regarding the interface head and connections with the cassette may found in co-pending, commonly assigned U.S. Provisional Patent Application No. 60/097,511 (Attorney Docket No. 16925-002200) filed on August 21, 1998, the full disclosure of which has been previously incorporated herein by reference for all purposes. The tube 100 is preferably held in place by a

threaded device 43 to hold the tube to the head 40. The threaded device 43 is not exposed to the fluid flow path as the tube 100 extends past the threaded device and prevents liquid contact with the device 43. Hence, an inert fluid pathway is maintained even though the device 43 may be made of materials such as stainless steel or iron which may corrode when exposed to reagents. Typically, the threaded device 43 has both internal and external threads so that it can be screwed into support 42 of the head 40. The head 40 also has a plurality of connectors 93 and 94 which preferably provide connections to delivery and vent lines of the system 10, respectively.

Fig. 7 shows a cross-section of the interface head 40 shown in Figs. 6 and 8. The opening 96 contains connector 93 which provides a connection with the valving and manifold on the delivery side as shown schematically in Fig. 14. In some embodiments, the holding tube or reservoir 206 may extend into the passage 97 shown in Fig. 7 from connector 93. Alternatively, the holding reservoir 206 may be either completely apart from passage 97 or passage 97 may be the entire holding tube and have a valve located at position 98. As seen in Fig. 8, the connectors 93 and 94 are typically offset from each other to be aligned with the tubular structures 100 and 102, respectively.

C. Fluid Delivery

Fig. 9 shows a preferred embodiment of the plumbing and fluid elements of a fully automated combinatorial synthesis system 10. As will be described, the plumbing and interface used with the present invention allows for precise delivery of reagents without diluting the fluids in the reaction vessel with solvents used as rinse fluids. Fig. 10 provides a simplified plumbing schematic of the system of Fig. 9 when the interface head 40 is engaged to eight reaction vessels 50. As can be seen, the syringe pumps 170 and 171 are used to provide delivery and/or extraction of fluids from the delivery side and the vent side of the reaction vessels 50.

Referring to Fig. 11, a simplified portion of the plumbing used in Fig. 9 will be described in detail. Fig. 11 shows one configuration used to deliver reagent to the reaction vessel. Typically, in some synthesis apparatus, as reagent is sent into the reaction vessel, it leaves a trail of small droplets or coatings of material as the bolus of reagent is sent through tubing to the target reaction vessel. Hence, if 1 ml of reagent were meant for delivery, some amount less than 1 ml would reach the final destination. Typically, a rinse fluid of solvent is delivered after the reagent delivery to clean the tubing and also to ensure than any amounts of reagent left in the tubing is picked up by the solvent rinse fluid and sent to reaction vessel. Introducing large amounts of solvent into the reaction vessel, however, is undesirable in some processes.

In the embodiment of Fig. 11, reagent is accurately metered into the reaction vessel without the introduction of rinse solvents into the reaction vessel. The general theory behind this concept is the use of a pump such as a syringe pump to push on an entire tubing or line filled with liquid, except for a small air or gas gap of about 0.22 ml to separate reagent and solvent portions. The air gap is introduced into the fluid bolus by valving which can switch the syringe pump to a gas source to add the air or gas into the line. The air gap is preferably included to prevent dilution between the reagent and solvent portions. Without the gap, for example, 400 microliters of reagents are required to deliver 200 microliters into the reaction vessel 50. With the gap, only 250 microliters are needed to deliver 200 microliters into the reaction vessel. The extra volume of reagent is used to fill the common passage between the holding reservoirs as discussed below.

This creates a substantially incompressible liquid column. This enables an accurate amount of reagent to be delivered into a holding tube or reservoir 206 immediately upstream from the reaction vessel 50. For example, if the syringe pump outputs 5 microliters, the substantially incompressible column should push 5 microliters into the

holding tube or reservoir 206. A valve 208 then seals off the holding tube from the liquid column in connecting passage 204. Then the reagent in the tube 206 is injected into the reaction vessel through gas pressure, typically after the connecting passage is cleared of liquid and fluidly coupled to a gas source. Hence, only non-diluted reagent of the amount desired is injected into the reaction vessel. Preferably, no solvent rinse fluid is delivered into the reaction vessel with the reagent. In one embodiment, the reservoir 206 is about 250 microliters, where any volumes in excess of 250 microliters flows directly into the reaction vessel before the reservoir valve 208 is closed.

Specifically, a syringe pump 170 is typically first used to prime the manifolds 172 and 174 with solvent. The manifolds are connected together with a passage 476. The passage may be designed to be large enough to be a reservoir. Solvent is first drawn into the syringe pump 170 as indicated by arrow 177. A valve 178 leading to the solvent source 179 is then closed. Solvent is then typically injected through the system to prime the manifolds 172 and 174. Reagent is then withdrawn via the syringe pump 170 using the solvent to help pull reagent as indicated by arrow 180. Once the desired amount of reagent is in the manifold, a valve 182 to reagent source 184 is closed. Although not required, a small air or gas gap G preferably exists between the solvent S and the reagent R as shown in Fig. 12.

The entire fluid column is then delivered under syringe pressure down towards the interface head 200 and the reaction vessels 50 as indicated by arrows 202. A manifold or block 203 is typically used to contain conduits such as connecting passage 204 and/or holding reservoirs 206. In addition to the reagent to be injected into the reaction vessels 50, the amount of reagent drawn from the reagent source 184 is preferably enough to fill slightly more than the connecting passage 204. Holding tubes 206 may be filled as desired based on the amount of reagent to be introduced for each reaction vessel. As shown in Fig. 19, the syringe pump 170 will meter reagent into the holding tube or reservoir 206

when reservoir valve 208 is open. The column of reagent R completely fills the common or connecting passage 204, the desired number of holding tubes 206, and extends slightly beyond the connecting passage 204. The syringe pump, connecting passage, and holding tubes are all illustrated in co-pending, commonly assigned U.S. Patent Application No. 60/063,134 (Attorney Docket No. 16925-001900) filed on October 22, 1997, the full disclosure of which has been previously incorporated herein by reference.

In this preferred configuration, the entire connecting passage 204 is then exhausted or blown into fluid waste container 210 via gas pressure from gas source 212. Once the connect passage 204 is cleared, the individual holding tubes 206 may then be pressurized to deliver the reagents into the reaction vessels. This may result from opening valve 208 and allowing gas to deliver reagent to the reaction vessels. In other embodiments, a pressure line may be connected directly to holding tube 206. After reagent delivery, the line or tubing upstream from the reaction vessel may be flushed with solvent to clean the flow path.

As shown in Fig. 9, the preferred embodiment of the synthesis apparatus has two syringe pumps 170 and 171. Pump 171 is used with an autosampler which can provide additional diversity reagents than those stored in containers R1-R9. When using pump 171 as the injection device, solvent may still be withdrawn from the solvent sources via tube 220. Additional reservoir or ballast tubes 222 and 224 may be used with the system. It should also be understood that other types of pumps may also be used besides a syringe pump. On such pump is described in co-pending, commonly assigned U.S. Patent Application No. 60/063,137 (Attorney Docket No. 16925-002010) filed May 29, 1998, the full disclosure of which is incorporated herein by reference for all purposes. In other embodiments, the fluid delivery system may use plumbing different than those shown in Fig. 9. Preferably, these other embodiments would be able to separate some portion of the reagent plug or bolus, either the leading edge portion or some portion in the middle. Once isolated, gas pressure can be

used to inject the isolated portion of reagent towards to desired reaction vessel. Preferably, the distance to be traveled by the isolated reagent bolus is small, between about 0.01" to 0.60" inches. In some embodiment, the holding tube 206 may not be needed if the isolation portion is the connecting passage 204. The system may comprise of a tubing, a valve upstream of the tubing, a pressurized gas source, and a waste vent in some embodiments. The waste vent would be connected and switched at the valve.

In some applications, it is desirable to use a gaseous reagent in the reaction vessel 50. The sealable reaction vessels 50 allow for the use of gaseous reagents, particularly under positive pressures. Unlike conventional septum-based systems, the cassette and reaction vessel configuration of the present invention allows the reaction vessel to be charged with gas and then sealed to process at the desired pressures. Openings created in septum systems will leak at positive pressures. The present system may also be closed after delivery at positive pressures such as 5 to 10 psi above ambient pressure or have a low pressure supply continuously flow and charging the vessel with gaseous reagent. The positive pressure may be required to get the desired molecular concentration of elements in the reaction vessel 50. At ambient pressures, the number of molecules may not be sufficient for synthesis.

D. Agitation Thermal Unit (ATU)

Referring now to Fig. 15A, the present invention is preferably based around the cassette 110 which contains the reaction vessels 50 used for combinatorial chemistry synthesis. The cassette 110, which may be designed to hold various numbers of reaction vessels, advantageously allows for the injection and extraction of liquids, reagents, or fluid washes from a reaction vessel 50 while maintaining an inert environment. Further details regarding the cassette 110 can be found in co-pending, commonly assigned U.S. Patent Application No. 09/095,731 (Attorney Docket No. 16925-001710)

filed on June 10, 1998, the full disclosure of which has been previously incorporated herein by reference for all purposes.

The reaction vessels 50 used in the present invention typically have a relatively small or moderate volume such as about 5 ml. This is due in part to the relatively small volume of reagents used per reaction vessel when generating combinatorial chemistry libraries. This minimizes total cost of synthesis. Furthermore, large volumes of resulting product are typically not necessary until the product has been analyzed for beneficial or desired properties.

Preferably, the reaction vessels 50 also contain tubing (Fig. 2A) which extend to the bottom of the reaction vessel. Because it is preferred that the cassette 110 be able to mate with an interface head 40 as previously described, the reaction vessel 50 is not open ended at its bottom surface and all connection are preferably located on upper ports 404 of the cassette 110. The tube or tubes inside the reaction vessel allows for the removal of liquids through the upper ports 404.

Accordingly, the relatively small size of the preferred embodiment of the reaction vessel 50 and the presence of tubing within the reaction vessel makes it difficult to use agitation devices placed within the reaction vessel. Such device include various shaped items which are moved in an axially reciprocal manner by an external magnet. The small stroke displacement allowed and the interference from internal tubes limits the effectiveness of such items placed in the reaction vessel.

In the preferred embodiment of Fig. 15A, the cassette 110 is connected to an agitator 410 which reciprocates the entire cassette as indicated by arrows 132. The motion is typically a lateral reciprocating motion but it should be understood that a variety of other motions such as circular or even vertical may be used. The agitator 410 of Fig. 15A is preferably a step motor 140 (Fig. 5) coupled to a cam mechanism 130 where the step motor can provide precise positioning of the cassette when the cassette comes to a stop.

This is particularly desirable in automated synthesis systems where a consistent stopping position is required so that a robotically controlled interface head can mate with the cassette. The system 10 may have sensors such as optical sensors mounted on the cassette 110, the cam 130, the interface head 40, and/or the ATU 468 to confirm the final position of the cassette. In manual operations of the interface head 40, a less accurate motor may be used for agitation since final position is less important as the user visually aligns the interface head with the cassette 110. Further details regarding the agitator can be found in co-pending, commonly assigned U.S. Provisional Patent Application No. 60/063,134 (Attorney Docket No. 16925-001900) filed on October 22, 1997, the full disclosure of which is incorporated herein by reference for all purposes. The motor generally reciprocates the cassette at a rate between about 100 and 300 strokes per minute, preferably about 250 strokes per minute. The total stroke displacement may be between about 0.25 and 0.75 inches, preferably about 0.5 inches. Some embodiments may agitate that cassette at 0.25 inches per side.

Chemicals used in solid phase and solution phase synthesis are sometimes volatile and unstable at room temperature. Accordingly, many of the synthesis operations occur at other temperatures to obtain the desired characteristics. In one embodiment, the reaction vessels 50 of the present invention may be operating in a temperature range from about -40°C to 150°C. These temperature changes are preferably attained by using a heated or cooled gas that is diffused into the hollow underside 430 of the cassette 110 as indicated by arrows 432. Although a variety of gases may be used, it is preferred that the gas is dry to prevent ice formation on the cassette. To stay within a ±5°C temperature variance, the dispersion of gas into the hollow underside 430 is maintained in a controlled fashion by a gas distribution plate 440 located below the cassette 110. The distribution plate 440 typically does not agitate with the cassette 110. The plate 440 has a plurality of holes 442 positioned in a grid pattern shown in Fig. 15B. The grid pattern varies with

the number of reaction vessels 50 in the cassette 110. In some embodiments, there is approximately one hole for each reaction vessel. The holes are typically circular with a diameter between about 0.05 to 0.30 inches. They are typically about 0.6 to 1.2 inches apart, again depending on the number of reaction vessels 50 present.

The gas or dry air is typically recirculated and pumped around using a fan 450. The fan 450 pulls air or gas down through vent 452 in the plate 440. The air or gas is then circulated through the heating/cooling element 460 and distributed back into the underside 430 of the cassette 110. The recirculation of gas or air minimizes the energy needed to maintain a desired temperature. The heating/cooling element 460 preferably contains tubing 462 for liquid nitrogen or other coolant. The element 460 also contains a heating element, typically resistive heating wire such as a kapton heater, to increase the gas or air temperature. The element 460 is typically made of a thermally conductive material such as aluminum. Additional plates of conductive material may be attached to the element 460 to increase thermal conductivity. In the preferred embodiment, fins 464 in a mostly hollow heating/cooling element 460 provides additional surface area for heat transfer with the circulating gas or air.

During agitation, the stroke displacement of the cassette 110 is such that the area above the plate 440 remains sealed or overlapped by the cassette or related part. Typically, the opening to the underside of the cassette 110 is larger than the opening exposing the cassette to heated or cooled gas to maintain enclosure during agitation. Although less desirable, heating using direct contact with the reaction vessels 102 may be designed with the cassette. Such a direct contact heater may require the cassettes to have enclosures for surrounding the surface of each reaction vessel or recesses for the reaction vessels formed in a solid block. Such a design may increase the weight of the cassette and also introduce additional thermal variances, such as insulation and expansion/contraction issues.

Referring to Fig. 15A, the combination of the agitator 410, the housing, the plate 440, the fan or gas recirculation unit 450, and heating/cooling element 460 form an agitation thermal unit (ATU) 468. When used with the cassette 110, this provides a system with allows for fluid injection/extraction, agitation, and temperature control all in one system in a cost efficient manner. The cassette 110 can be removed and replaced with another cassette so that the agitation and thermal process can continue while the other cassette awaits other processing. The plate 440 may also be designed to be replaceable so that even heat distribution can be achieved for cassettes of different sizes and numbers of reaction vessels 50. Insulation may also be provided around the ATU to minimize condensation and ice formation on the device. For example, insulative material may be placed on the interior of the side walls of the cassette.

Referring now to Fig. 16, a cassette fan 470 is preferably positioned to generate air or gas flow over the upper surface of cassette 110 during cooling procedures. The air or gas flow over the upper surface minimizes or eliminates moisture accumulation which can be difficult to remove from cavities 104 and 106. Moisture in these cavities may degrade organic reactions in the reactions vessels 50 if the moisture is not removed prior to injection or extraction of materials into or from the reaction vessels.

The integrity of the air/liquid seal with the reactions vessels 50 and the lid 111 of the cassette 110 further benefit from having a fan or gas flow generator 470 generating flow over the upper surface of the cassette 110. In some embodiments, the fan 470 is positioned to blow directly at the lid 111 of the cassette 110 or across the lid. At sub-freezing temperatures such as -20°C, the elastomeric material 472 (Fig. 2A) forming a seal between a cap valve 474 on the reaction vessel 50 and the lid 111 of cassette 110, hardens and no longer guarantees seal integrity. Typically, the hardened elastomeric material 472 will not be sufficiently compliant to reshape and form a seal with the upper surface of the cap valve 474 when the cap valve is rotated to open or

close access to the reaction vessel 50. This non-compliance may allow for air or liquid to seep past the elastomeric material 472 and contaminate the reactions in the reaction vessels 50. The elastomeric material 472 and cap valve 474 are described in commonly assigned, copending U.S. Patent Application Serial No. 09/095,731 (Attorney Docket No. 16925-001710) filed on June 10, 1998, the complete disclosure of which has been previously incorporated herein by reference. The flow of ambient air or gas across the cassette 110 prevents the lid and the elastomeric material 472 from dropping lower than -20°C. Although it is usually sufficient to flow ambient air across the cassette 110, in some alternative embodiments, it may be desirable to blow heated air or gas across the lid 111. The flow of ambient air allows the elastomeric material 472 to remain sufficiently compliant even if the temperature in the reaction vessel 50 is lower than -20°C. The reaction vessel 50 is sufficiently insulated from the lid 111 and much closer to the source of cooling so as not to be affected by the air flow over the lid. The prevention of condensation contamination and loss of seal integrity ensures the contamination-free performance of the system 10 during synthesis.

E. Reliability

The system 10 according to the present invention preferably includes devices for ensuring that liquid delivered from the syringe pumps 170 and 171 actually reach the reaction vessels. Since the nature of combinatorial synthesis involves creating substances from many different combinations of materials, the unknown nature of the reactions which may occur may result in precipitates or solids coming out of solution and blocking the fluid lines connected to the reaction vessels 50. With these synthesis apparatus typically running 24-hours a day under automated control, errors may occur in the middle of the night, without personnel around to respond to the error. This may create problems such as fluid lines backed up with reagents (which is costly) or may result in lost

processing time. The reliability aspect of the present invention provides devices and methods for detecting non-delivery of reagents or solvents and preferably devices to troubleshoot the problem.

Referring to Fig. 17A, a simplified system comprising of a reaction vessel 50 and a metering system 580 is shown. Fig. 17A shows that a plug or bolus 582 of reagent is being delivered to the vessel 50 by pressurized gas as indicated by arrow 584. The gas from source 586 flows behind the bolus 582 and is typically flowing at a constant rate at this time. When the bolus 582 of reagents is delivered into the vessel 50, as shown in Fig. 17B, the resistance in front of the gas is no longer there the gas indicated by arrows 584 flows at a much higher rate towards vent 588. Hence, when reagents or solvents are delivered, there is a signature increase or spike in the gas flow as shown in Fig. 17C. This graph of gas flow rate versus time shows that there is a constant flow rate 590 when the reagent is being delivered and then an increase 592 in flow rate once delivery is successful. If the fluid path to the vessel 50 is blocked by precipitates or other solids, the signature increase in flow rate will not appear. Once a blockage is detected, a number of different action may be taken. Depending on where the blockage is located, the controller may notify a user that an error has occurred or the controller may simply decide not to use that reaction vessel 50 until processing is completed.

Furthermore, the line to the vessel 50 may also have an optical liquid sensor which can detect the passage of fluid through the delivery line. The sensor uses a photosensor to register the changes in the index of refraction of the transparent or translucent delivery line. Of course, it should be understood that the flow meter may be installed along other portions of the system to detect delivery of material thorough a particular line.

In a preferred embodiment, as shown in Fig. 9, a flow meter 550 monitors fluid, typically gas flow, from locations 552, 554, 556, and 558. The gas flow measured at these locations can be used to determine whether chemicals

have been properly delivered to their destinations. For example, the failure of proper gas flow to reach point 558 on the vent side of valves connected to the interface head 500 would indicate a failure to deliver the reagent or solvent wash into the reaction vessel 50. Similar conclusions can be made based on low to no gas flow readings at the other locations. Position 552 reads delivery to manifold 172, position 554 reads delivery from manifold 172 to manifold 174, and position 556 read output from the autosampler 560. Additional sensors may also be placed at different locations along the flow path.

Furthermore in Fig. 14, liquid sensors 570 using optical or other detection methods are installed along the flow path to verify that solvent or reagent has actually reached the desired locations. Preferably, the detection methods are non-invasive and will not contaminate the environment of the flow path. The sensors 570 are preferably located along the holding tubes 206 and also at the end of the connecting or common passage 204. The liquid sensor 570 located at the end of the connecting or common passage 204 (i.e. a common passage liquid sensor) serves to simplify the operation of the syringe pump 170 during fluid delivery. Instead of having to know the exact volume to push from the syringe pump 170 to fill the connecting passage 504, the syringe pump operates until the sensor 570 detects the presence of fluid at the end of the connecting passage 504. This simplifies operation and possible slippage from the liquid column being pushed to the interface head. It should be understood, of course, that the sensors 570 may be located at a variety of positions along the fluid flow path such as near the reagent sources and near the waste containers.

H. Interface Head Movement

In exemplary embodiments, the system 10 uses a specific control algorithm to minimize the motions of the interface head 40. Since the coupling and decoupling of the interface head 40 with cassette 110 typically requires the

filling of connecting or common passage 204 which is then discarded, excessive interface head movements result in wasted reagents which can be costly. To minimize movements and also to reduce time use to create combinatorial libraries, the interface head motion may be based on a Hilbert curve motion. The Hilbert curve is a recursion process that is used to solve the positioning of desired products on the cassette and also to control head motion.

In some embodiments, the Hilbert curve is used prior to synthesis to determine where the desired products should be located to minimize head motion. In other embodiments, the Hilbert curve algorithm is used to determine head motion if the location of the products of each reaction vessel is pre-determined. The algorithm is typically used before the head starts to move, but alternatively, the algorithm may be activated during processing. It should be understood that the automated system 10 may also be used in combination with a hand held apparatus to minimize the number of locations the Hilbert curve needs to solve for if manual operation will simplify calculations. Such a manual interface head is described in co-pending, commonly assigned U.S. Provisional Patent Application No. 60/097,511 (Attorney Docket No. 16925-002200) filed on August 21, 1998, the full disclosure of which has been previously incorporated herein by reference for all purposes.

In the present invention, the combinatorial libraries contain most or all combinations of the reagents organized by groups called dimensions. For example, reagents A and B might be combined with reagents 1, 2, and 3, to produce compounds A1, A2, A3, B1, B2, and B3. The reagent collections are organized into steps, or dimensions, each consisting of a subset of the reagent collection. The previous example used reagents A and B in dimension one and reagents 1, 2, and 3 in dimension two.

The term reagent space is used herein to refer to the n-dimensional information space comprising the combinations of reagents from each of n dimensions. Each

combination of reagents corresponds to one compound and occupies one vertex in the reagent space.

As described, the system 10 contains a series of vials or reaction vessels 50 into which the reagents are dispensed. The reaction vessels 50 are grouped, preferably 48 per cassette 110. The system 10 stores the reagents and delivers them to the reaction vessels through a plumbing system comprising reagent storage containers, electronically controlled valves, flow sensors, tubing, and a robotic mechanism.

To reduce the time to build a library, it is desirable to minimize the amount of rinsing of reaction vessels while still observing the compatibility rules of the reagents and solvents. To reduce the time to build a library, it is also desirable to minimize the amount of mechanical motion of the fluid head.

A robotic mechanism containing a fluid interface head 40 connects the tubing that delivers the reagents, to the reaction vessels. When the fluid head 40 is connected to a cassette 110, the plumbing system has access to eight reaction vessels 50. The fluid interface head 40 must be disconnected, moved, and reconnected to gain plumbing access to other vessels 50. In operation, the system 10 delivers a reagent to all reaction vessels on a cassette 110 that need it before the plumbing is rinsed and the next reagent is delivered. After all reaction vessels on a cassette receive their deliveries, the next cassette is similarly processed.

In order to minimize rinsing and mechanical motion, a key factor is the manner in which compounds are assigned to reaction vessels. In mathematical terms, this is a mapping between reagent space and reaction vessel space. What is needed is a mapping between the vertices in the n-dimensional reagent space and the one-dimensional reaction vessel space.

Mathematician D. Hilbert devised the Hilbert Space-Filling Curve which has useful properties for the present application. A space-filling curve is a mapping between one-dimensional space and n-dimensional space. In simple terms, it folds a line into n dimensions, somewhat like a string

could be routed to fill a plane or a volume. Given a point in one-dimensional space, it produces a vertex in n-dimensional space. The Hilbert curve is especially useful because of its clustering property, in that, as it fills the n-dimensional space, it tends to minimize excursions out of the local region that is filling at the moment. It is this clustering property that helps minimize rinsing and mechanical motion. The clustering property is further described in "Analysis of the Clustering Properties of Hilbert Space-Filling Curve", submitted to IEEE Transactions on Knowledge and Data Engineering, March 1996, the full disclosure of which is incorporated herein by reference for all purposes.

Using the algorithm in Arthur R. Butz's "Alternative Algorithm for Hilbert's Space-Filling Curve," IEEE Transactions on Computers, April 1971, pp. 424-426, the full disclosure of which is incorporated herein by reference for all purposes, a Hilbert space is constructed having the same number of dimensions as the reagent space in the target library. A Hilbert space is cubic in that there are the same number of coordinates along each dimension, as in 8 X 8 X 8. The number of coordinates in each dimension must also be a power of two. In constructing an Hilbert space suitable for library synthesis mapping, the reagent space dimensions are adjusted upward in size so that they are cubic, and so that each dimension contains a number of coordinates that is a power of two. For example, a 3 X 7 X 9 reagent space would require the generation of a 16 X 16 X 16 Hilbert space. This means that the desired reagent space is a sub-cube of the Hilbert space.

Each vertex in the Hilbert space is assigned to zero or more reaction vessels. A vertex is assigned to zero reaction vessels if it lies outside the sub-cube comprising the reagent space, or if the chemist has "pruned" the associated target compound from the library. A vertex is assigned to more than one reaction vessel if the chemist desires multiple instances of a compound.

The Hilbert space results in an ordering of all vertices, by virtue of its n-dimensional to one-dimensional

mapping. It enables the construction of a sequence of vertices that must be assigned to at least one reaction vessel. This list is then assigned, one-by-one to the list of available reaction vessels, leaving empty those reaction vessels that the chemist sets aside for controls.

For each dimension, there is a list of reagents. This list is ordered so as to minimize rinsing. This is done by partitioning the reagent list according to compatible solvent groups. For example, if a dimension uses reagents A, B, C, D, E, F, and G, it might be partitioned as {A, C, F), {B, G}, {D, E} if the reagents shown here in a bracketed group are compatible. Also, the partitions are ordered, resulting in an optimal reagent ordering. For example, if {B, G} would require an intermediate rinse if it followed {A, C, F}, a better partition ordering might be ({A, C, F}, {D, B}, {B,G}), yielding an ordered reagent list of (A, C, F, D, E, B, G). It is this ordered list of reagents that is assigned to the coordinates of the reagent space.

While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, the system may use a reagent delivery unit without extraction capability in place of a reagent delivery/vent unit. In alternative embodiments, the circumferential seal could be formed by an O-ring of sufficient circumferential contact area to seal against a tubular structure. As seal of non-circular shape, but in contact all around the tubular structure may alos be used. Reaction vessels of different sizes and shapes may also be used. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

WHAT WE CLAIM IS:

1. A system for the synthesis of combinatorial chemical libraries, said system comprising:

a reagent delivery/vent unit; and

a chemical processing unit containing a plurality of reaction vessels, wherein said reagent delivery/vent unit has a plurality of tubular structures adapted to slidably engage cavities in said chemical processing unit adapted to receive said tubular structures to form a circumferential seal about at least one of said tubular structures.

2. A system as in claim 1 wherein said tubular structures are non-piercing tubular structures.

3. A system as in claim 1 wherein said reagent delivery/vent unit comprises a fluid interface head containing said plurality of tubular structures.

4. A system as in claim 1 wherein said chemical processing unit comprises a cassette which contains said plurality of reaction vessels and defines said plurality of cavities adapted to receive said tubular structures to form said circumferential seal about each of said tubular structures.

5. A system as in claim 4 wherein said cassette comprises a plurality of valves, said valves adapted to control flow through a fluid pathway between at least one of said tubular structures of the reagent delivery/vent unit and at least one of said reaction vessels in said cassette.

6. A system as in claim 4 wherein said valve is movable to a position forming a fluid connection between at least two of said tubular structures and sealing said at least two tubular structures from the reaction vessels.

7. A system as in claim 1 wherein said reagent delivery/vent unit comprises a plurality of fluid pumps and reagent sources.

8. A system as in claim 1 wherein said chemical processing unit comprises a cassette containing said plurality of reaction vessels, said cassette mounted on an agitation thermal unit for processing reagents delivered into said reaction vessels.

9. A system as in claim 8 further comprising a gas flow generator positioned to circulate gas over an upper surface of the cassette to maintain said upper surface above about -20°C.

10. A system as in claim 1 wherein said tubular structures and said cavities in said chemical processing unit have a side wall interference between about 0.003 inches to 0.006 inches.

11. A system as in claim 1 wherein one of said tubular structures forms a distal end seal with one of said cavities.

12. A system as in claim 1 wherein said reaction vessels are adapted to maintain a seal at above atmospheric pressures.

13. A system for the synthesis of combinatorial chemical libraries, said system comprising:
a reagent delivery unit;
a chemical processing unit; and
a connector adapted to fluidly couple said units together with an air/liquid-tight seal, the connector including a tubular structure and a support defining a cavity for receiving said tubular structure.

14. A system as in claim 13 wherein said tubular structure forms a distal end seal with said cavity.

15. A system for delivering reagents into a reaction vessel for the synthesis of combinatorial chemical libraries, said system comprising:

a removable interface head fluidly couplable to said reaction vessels to define a fluid pathway into said reaction vessel;

a holding reservoir downstream from a pump source, upstream from said reaction vessel during reagent delivery, and adapted to move together with said interface head, said reservoir having an inlet and an outlet;

a reservoir valve located near said inlet of the holding reservoir to control flow into the reservoir; and

a pressurized gas source fluidly couplable to said reservoir.

16. A system as in claim 15 comprising a plurality of holding reservoirs, said reservoirs fluidly coupled together by a common passage.

17. A system as in claim 15 further comprising:

a reagent source adapted to be fluidly coupled to said valve;

a solvent source adapted to be fluidly coupled to said valve; and

a syringe pump adapted to be fluidly coupled to said valve.

18. A system as in claim 15 further comprising a syringe pump adapted to deliver a fluid bolus comprising a reagent portion and a solvent portion, said fluid bolus extending from said holding reservoir to said syringe pump after delivery.

19. A system as in claim 18 wherein said fluid bolus has an air gap of less than 0.22 ml between said reagent portion and said solvent portion.

20. A system as in claim 18 wherein said pressurized gas source is adapted to flow the fluid in the holding reservoir into the reaction vessel.

21. A system as in claim 15 wherein said holding reservoir has a liquid sensor adapted to register the presence of liquid.

22. A system as in claim 15 wherein said holding reservoir is coupled to a common passage which is fluidly coupled to said pressurized gas source and a fluid waste container.

23. A system as in claim 22 wherein said common passage has a common passage liquid sensor adapted to detect when the common passage is completely filled.

24. A system as in claim 15 wherein said interface head is adapted to be fluidly coupled to at least eight reaction vessels.

25. A system as in claim 15 where said interface head has at least two tubular structures to slidably engage a cassette containing said reaction vessel.

26. A system as in claim 25 wherein one of said tubular structures is fluidly coupled to said holding reservoir and another of said tubular structures is fluidly coupled to a vent manifold.

27. A system as in claim 15 further comprising a flow sensor coupled to said pressurized gas source, said

sensor adapted to detect flow of pressurized gas delivering fluid into the reaction vessel.

28. A method for minimal dilution of a reagent used in the synthesis of combinatorial chemical libraries comprising:

forming a substantially incompressible fluid bolus comprising a reagent portion and a solvent portion;

pushing said substantially incompressible fluid bolus with a pump to meter a desired amount of reagent into a holding reservoir, said amount corresponding to the amount to be delivered into a reaction vessel; and

sealing said holding reservoir from the pump; and

using a pressurized gas source to blow said desired amount of reagent into the reaction vessel.

29. A method as in claim 28 wherein said fluid bolus extends from the pump to a holding reservoir valve before being pushed into the holding reservoir.

30. A method as in claim 28 further comprising ejecting to a waste container, a portion of the fluid bolus in a fluid conduit upstream and adjacent to the holding reservoir; and

fluidly coupling said pressurized gas source to the fluid conduit.

31. A method as in claim 28 further comprising flowing regent that overfill the holding reservoir into the reaction vessel.

32. A method for minimizing mechanical motion in the synthesis of combinatorial chemical libraries using a plurality of reagents, the method comprising:

generating a Hilbert space by sizing upward a reagent space;

slidably engaging a fluid delivery system to a reaction vessel; and

delivering reagents into said reaction vessel based on said Hilbert space mapped into a reaction vessel space.

33. A method for minimizing mechanical motion in the synthesis of combinatorial chemical libraries using a plurality of reagents, the method comprising:

generating a Hilbert space by sizing upward a reagent space;

ordering the reagents by analyzing their compatibility groups;

assigning one reagent to each coordinate of each dimension in the reagent space; and

mapping the Hilbert space into a reaction vessel space while observing rules for controls, empty reaction vessels, and replicates.

34. An apparatus for use with a cassette containing a plurality of reaction vessels for the synthesis of combinatorial chemical libraries, said apparatus comprising:

a heat exchanger;

a gas recirculation unit adapted to flow gas towards the heat exchanger and supply heat-exchanged gas around the reaction vessels; and

an agitator adapted to reciprocably agitate the cassette and the reaction vessels therein.

35. An apparatus as in claim 33 further comprising a gas distribution plate adapted to evenly distribute heat-exchanged gas around said reactions vessels within a temperature variance of ±5°C

36. A method for testing the successful delivery of liquid in a synthesis apparatus comprising the steps of:

flowing liquid towards a target site;

flowing gas to move said liquid to the target site; and

determining if there is an increase in gas flow rate.

37. A method for the synthesis of combinatorial chemical libraries comprising:

slidably engaging a fluid connector between a reagent delivery unit and a chemical processing unit, wherein a tubular member of the fluid connector passes through a patent opening;

forming a circumferential seal about said tubular member;

flowing chemicals through said tubular structure from said reagent delivery/vent unit to the chemical processing unit.

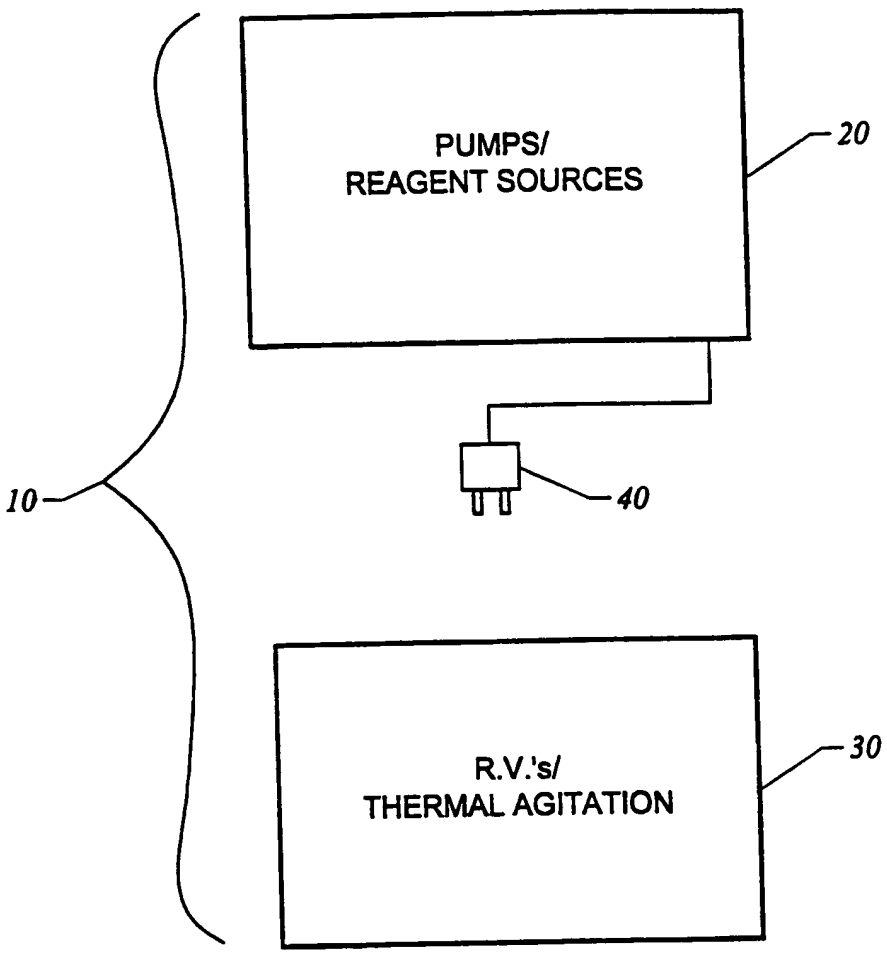

FIG. 1A

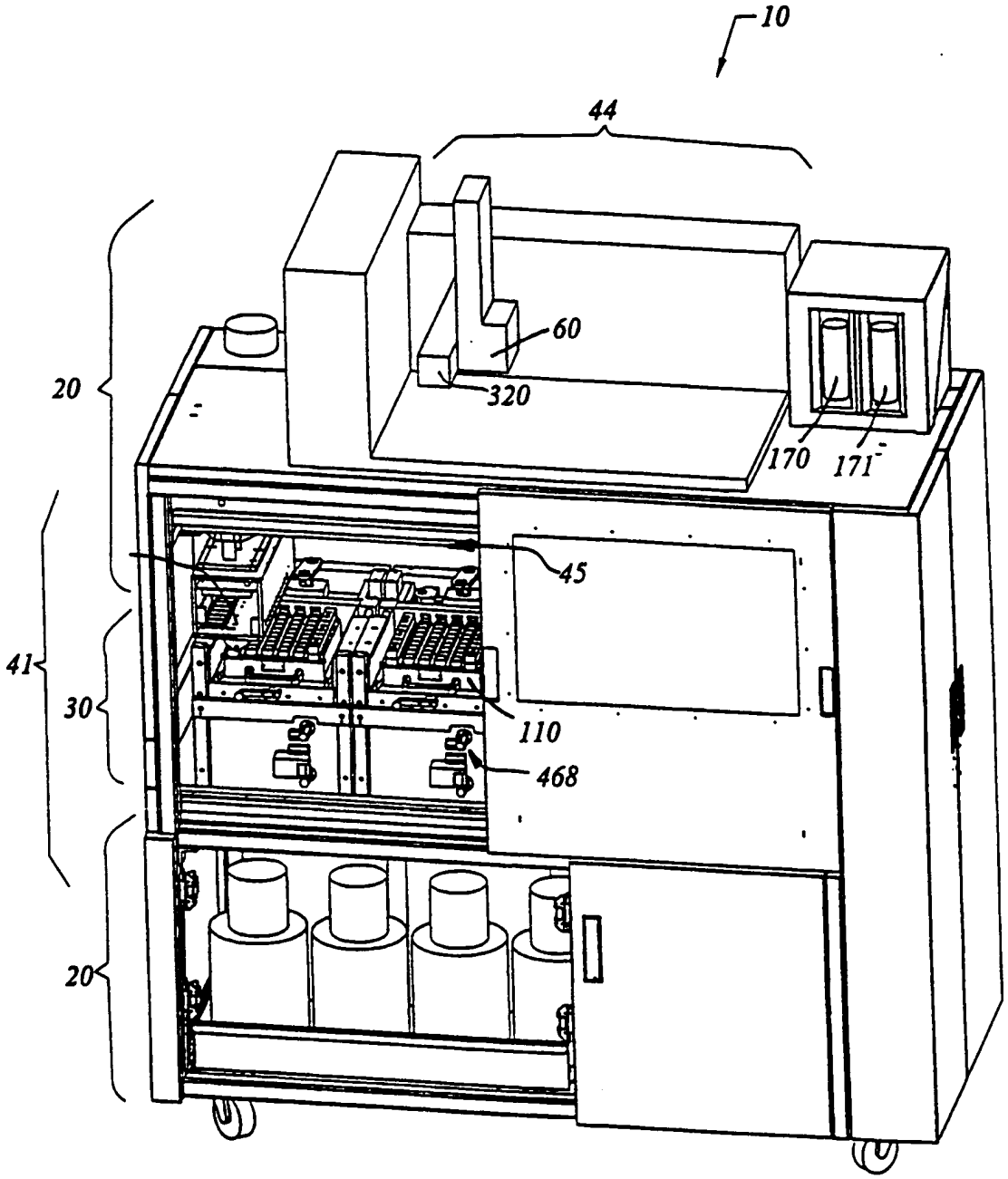

FIG. 1B

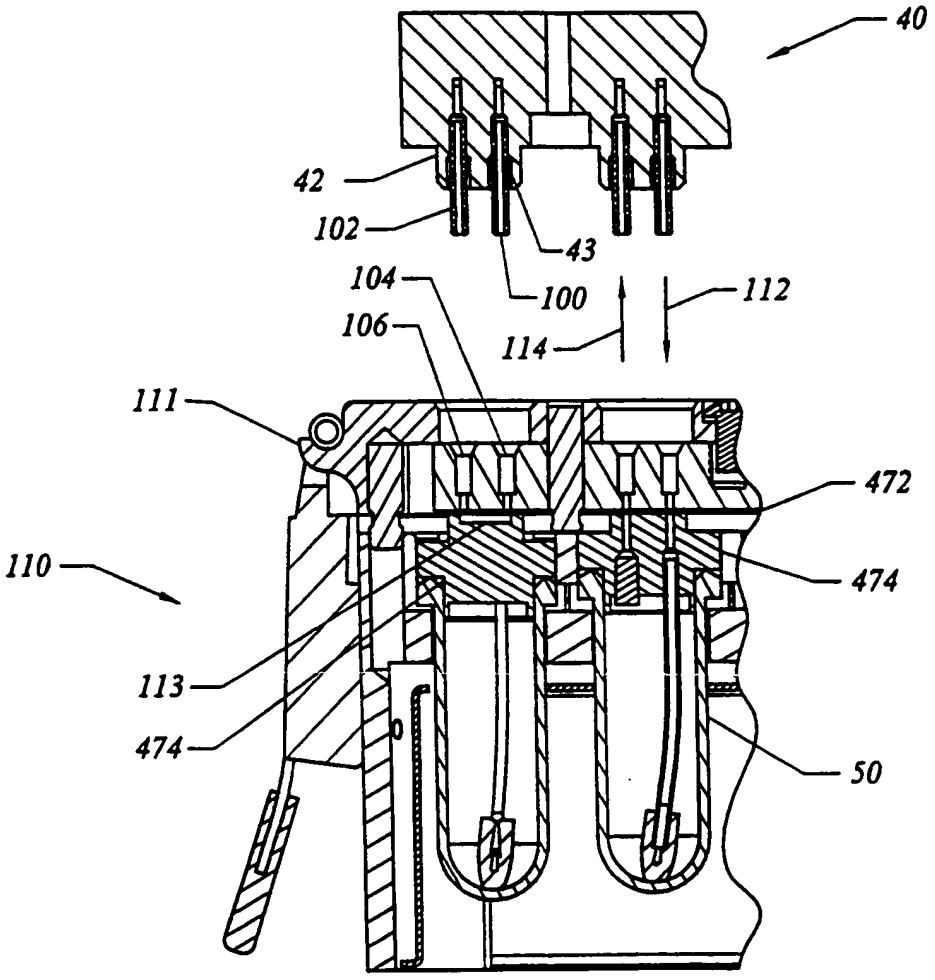

FIG. 2A

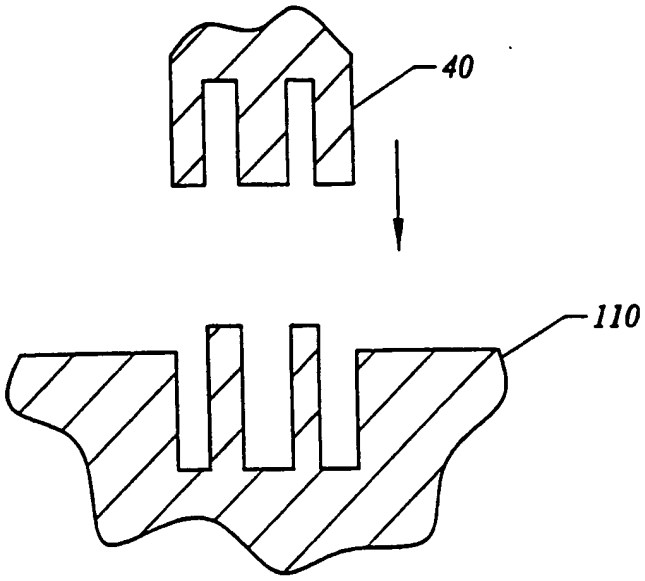

FIG. 2B

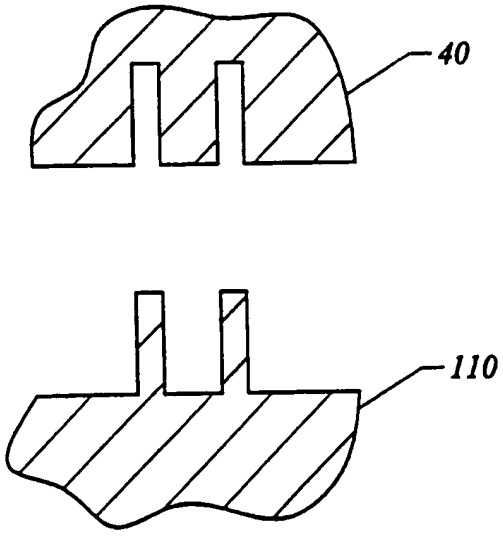

FIG. 2C

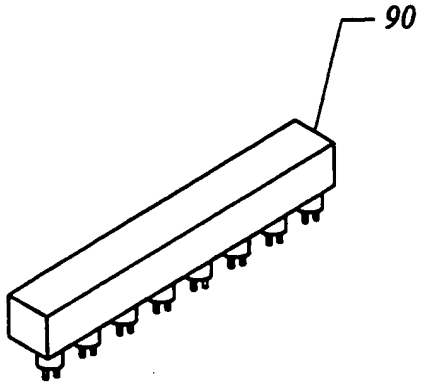

FIG. 3A

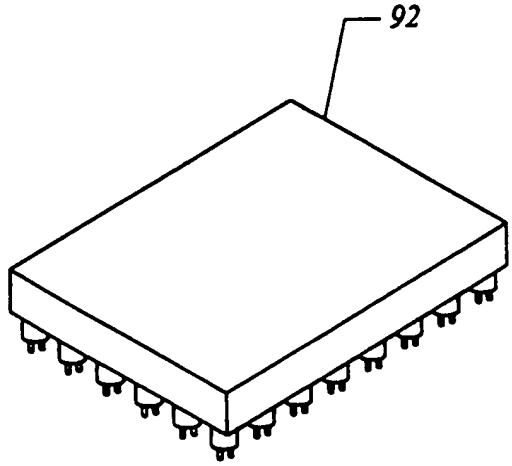

FIG. 3B

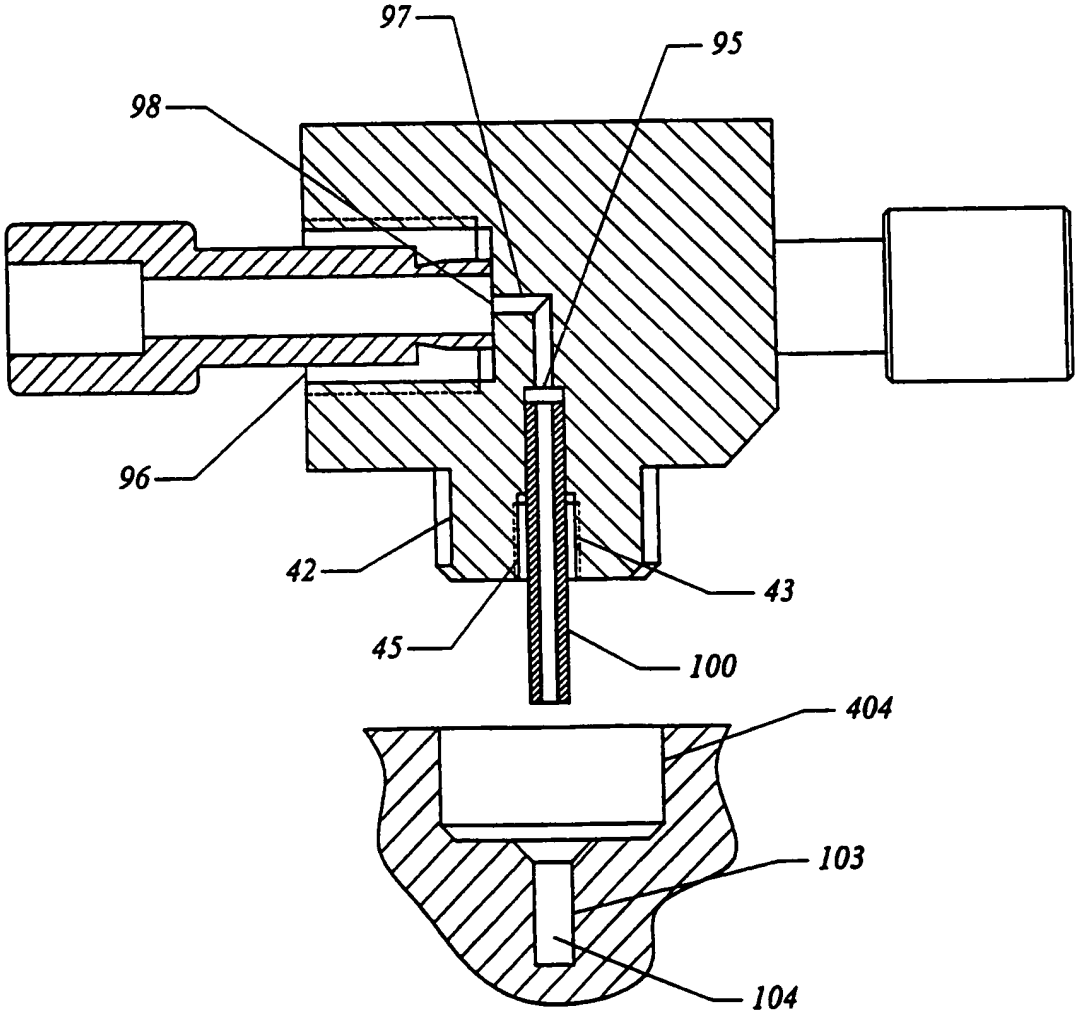

FIG. 4

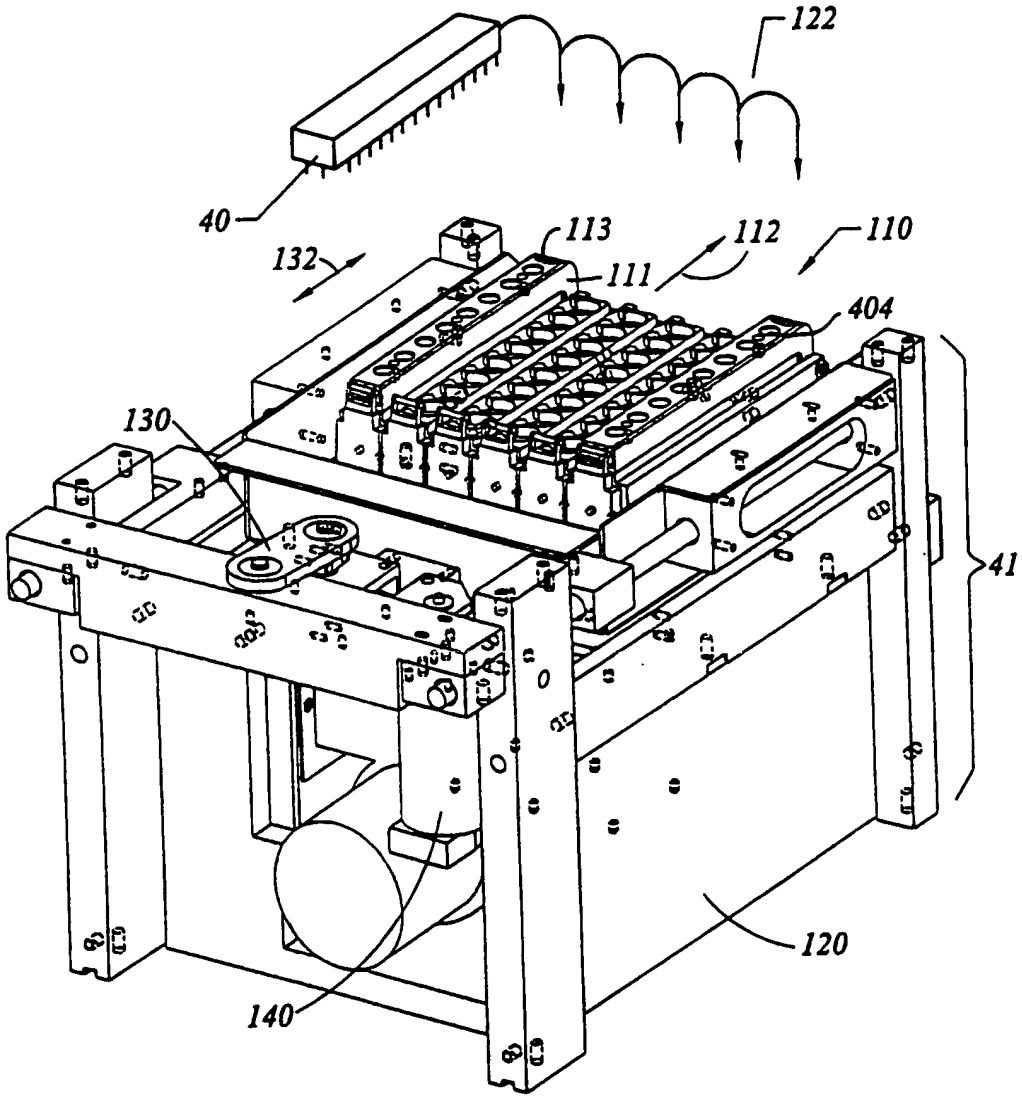

FIG. 5

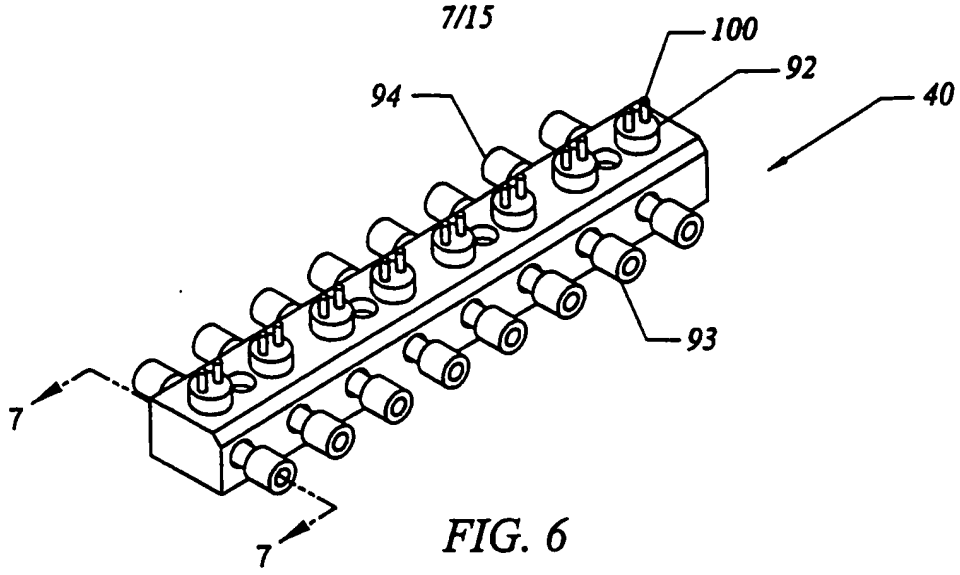

FIG. 6

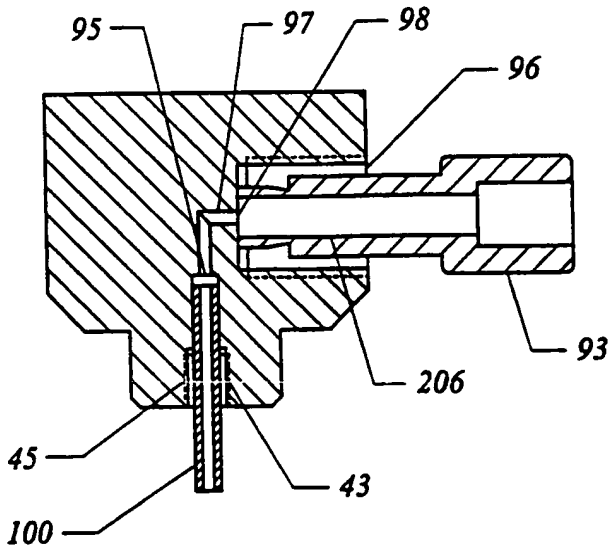

FIG. 7

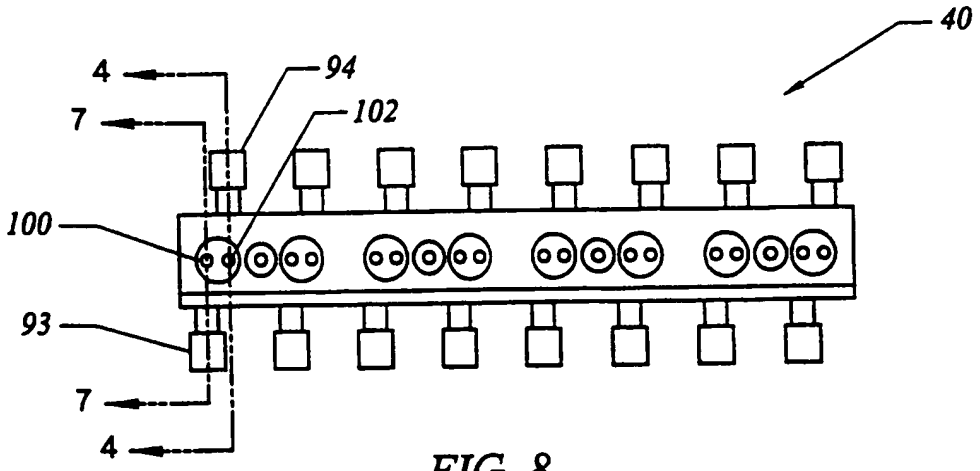

FIG. 8

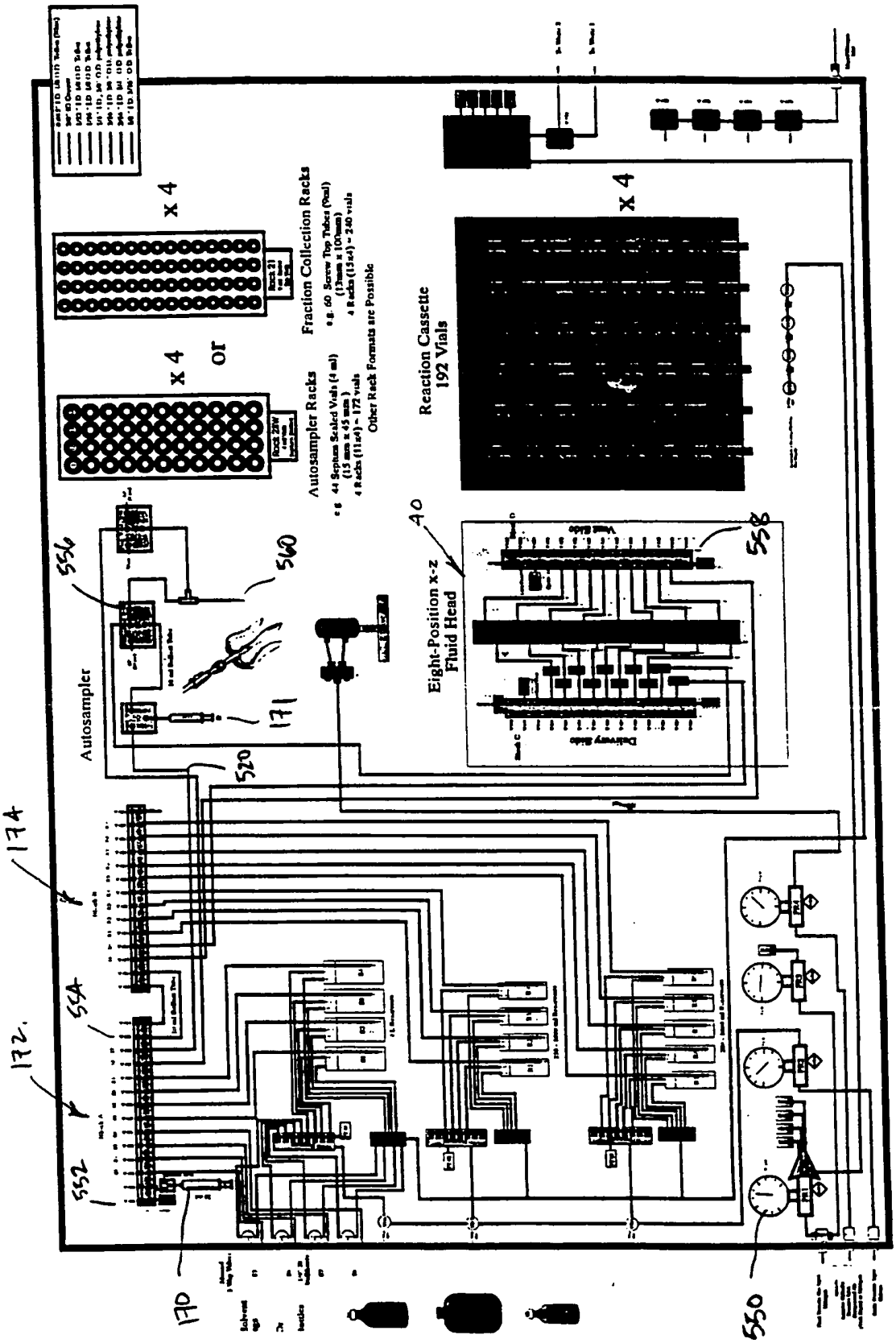
x 4
x 4
or
Fraction Collection Racks
Autosampler Racks
Other Rack Formats are Possible
x 4
Reaction Cassette
192 Vials
40
Eight-Position x-z
Fluid Head
Autosampler
174
172
554
552
170
530
560
556
558
520
171

FIG_9

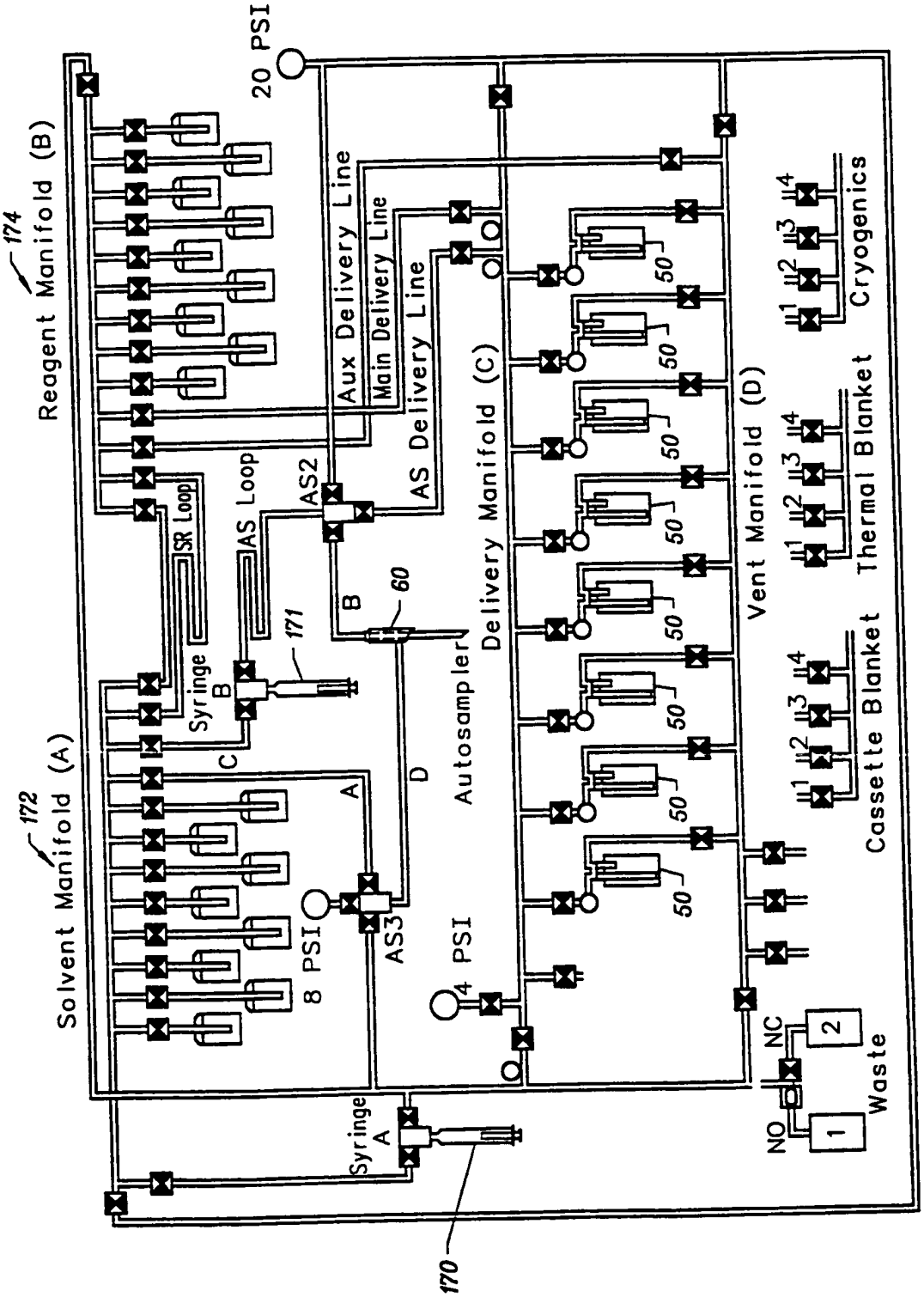
Reagent Manifold (B)
174
Solvent Manifold (A)
172
20 PSI
Aux Delivery Line
Main Delivery Line
AS Delivery Line
Delivery Manifold (C)
Vent Manifold (D)
Cryogenics
Thermal Blanket
Cassette Blanket
SR Loop
AS Loop
AS2
AS3
Syringe B
Syringe A
Autosampler
171
60
170
8 PSI
4 PSI
NC
NO
Waste
50

FIG. 10

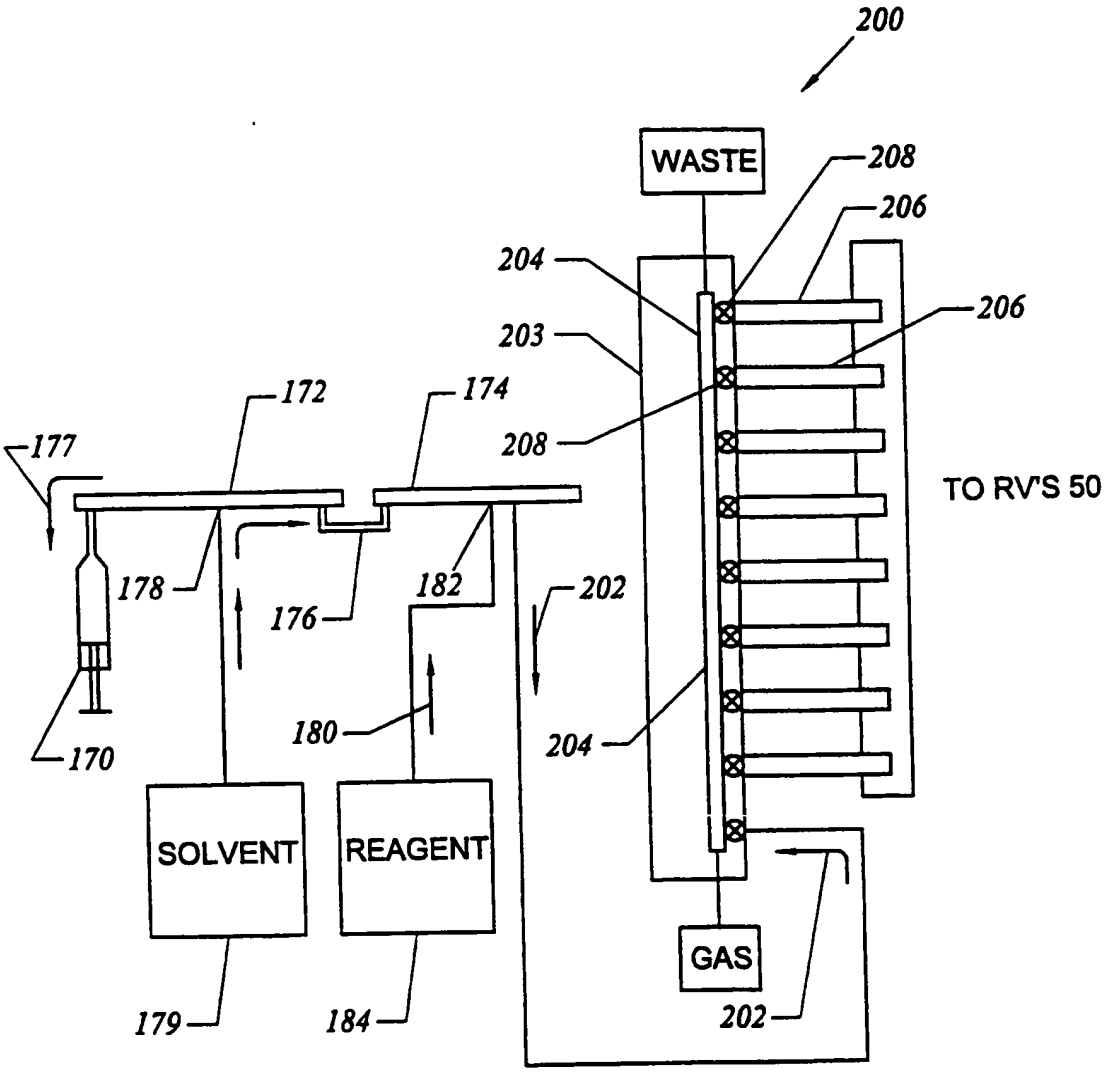
200
WASTE
208
206
204
206
203
172
174
177
208
TO RV'S 50
178
176
182
202
180
204
170
SOLVENT
REAGENT
GAS
179
184
202

FIG. 11

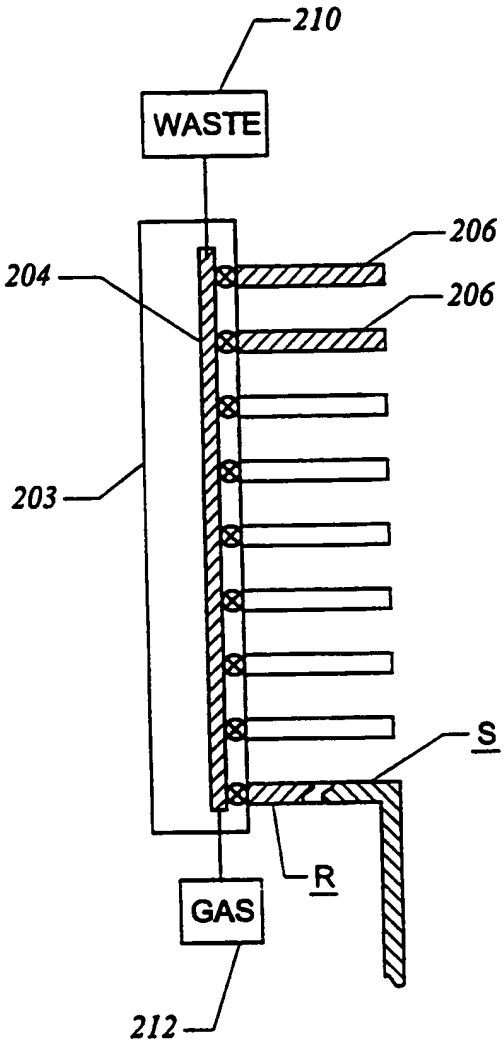

FIG. 13A

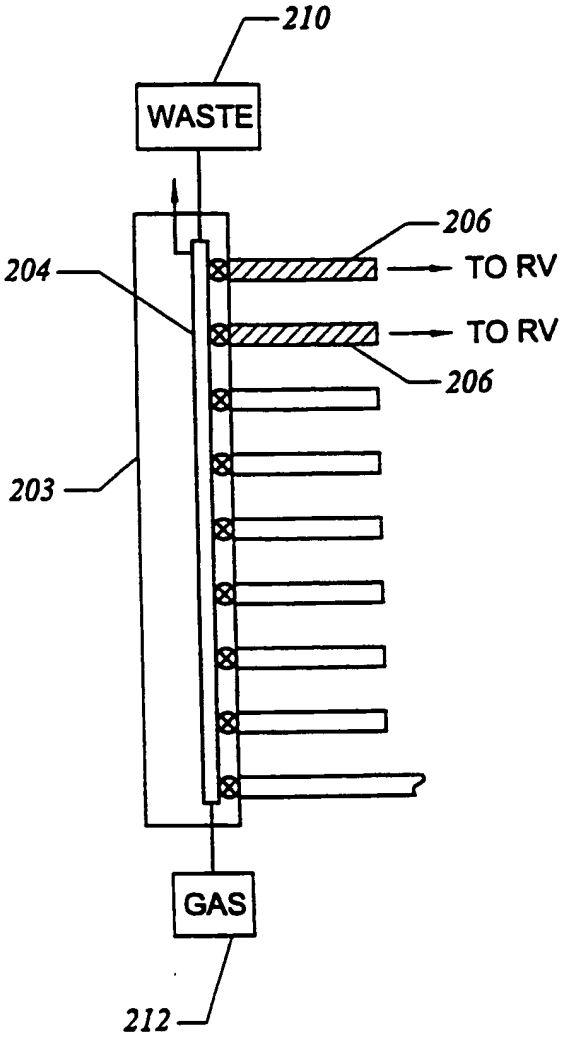

FIG. 13B

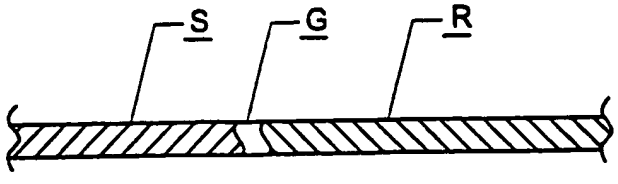

FIG. 12

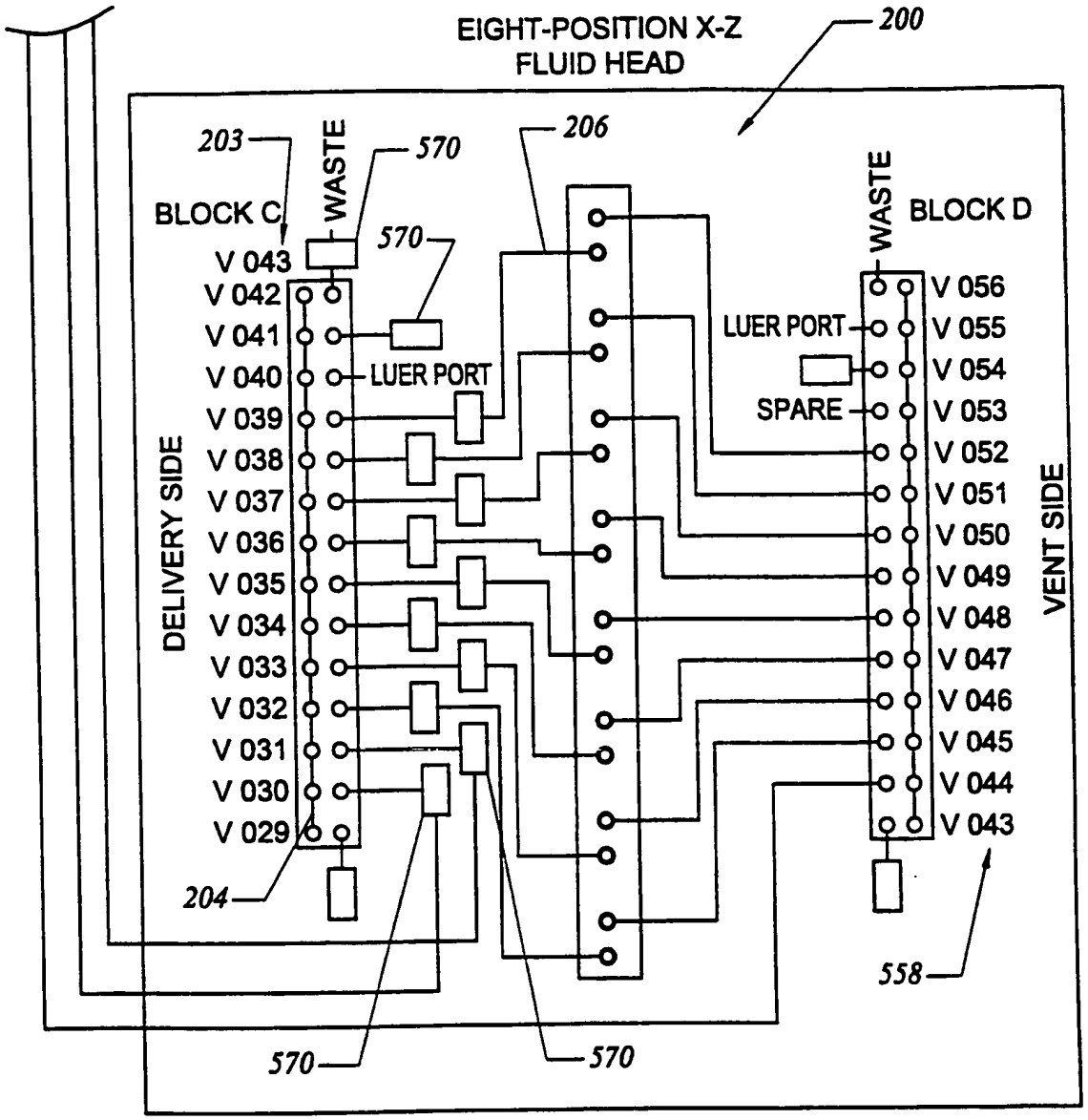
EIGHT-POSITION X-Z
FLUID HEAD
200
203
WASTE
570
206
BLOCK C
V 043
570
V 042
V 041
V 040
LUER PORT
V 039
V 038
V 037
V 036
V 035
V 034
V 033
V 032
V 031
V 030
V 029
DELIVERY SIDE
204
570
570
WASTE
BLOCK D
LUER PORT
SPARE
V 056
V 055
V 054
V 053
V 052
V 051
V 050
V 049
V 048
V 047
V 046
V 045
V 044
V 043
VENT SIDE
558

FIG. 14

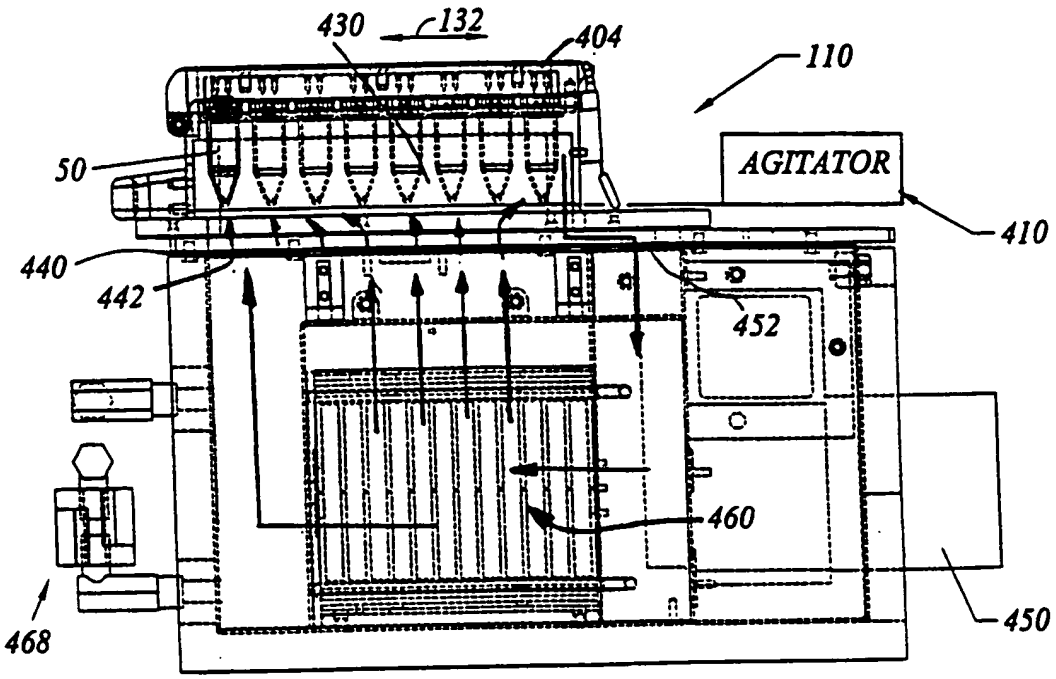

FIG. 15A

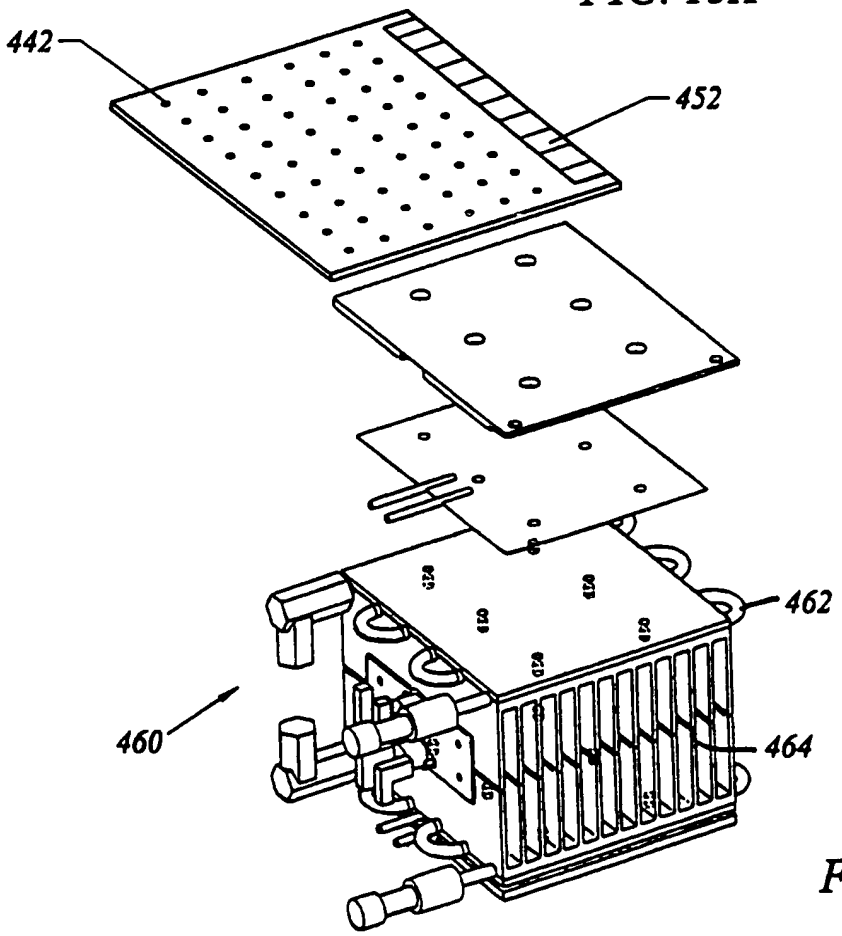

FIG. 15B

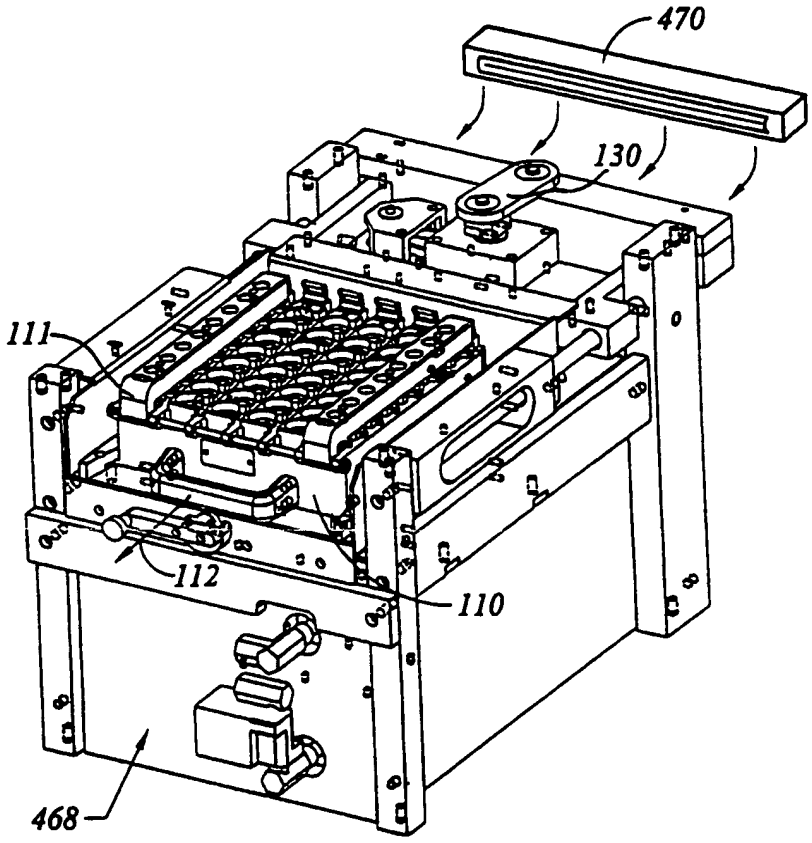

FIG. 16

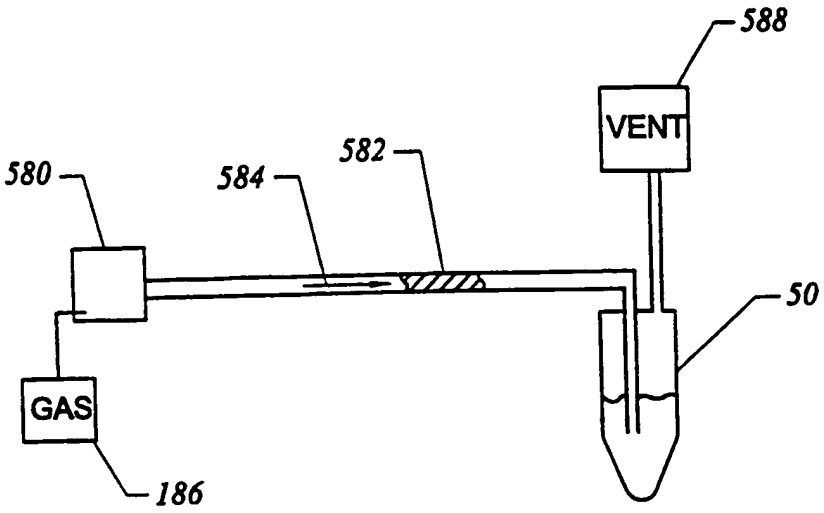

FIG. 17A

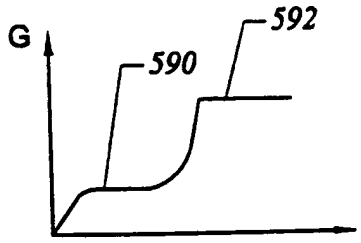

FIG. 17C

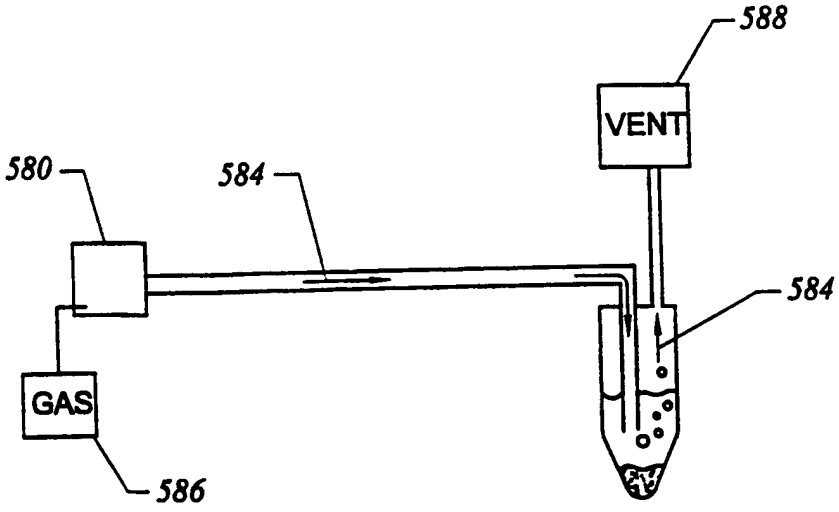

FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22193

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01L 3/02
US CL :422/100, 102, 103; 436/174, 180; 73/864.14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/100, 102, 103; 436/174, 180; 73/864.14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,746,491 A (OHLIN) 24 May 1988 (24.05.88), figures 4 and 8.	1-14, 37
X	US 5,019,348 A (OHMS ET AL) 28 May 1991 (28.05.91), figures 2 and 3.	1-4, 8-14, 37
X	US 5,660,792 A (KOIKE) 26 August 1997 (26.08.97), figures 4 and 5.	1-4, 7-14, 37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
01 FEBRUARY 1999	19 FEB 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JAN M. LUDLOW Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22193

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-14, 37

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22193

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-14 and 37, drawn to a chemical process apparatus and method of use.
Group II, claim(s) 15-31, drawn to a fluid delivery apparatus and method of use.
Group III, claim(s) 32-33, drawn to a method of minimizing motion.
Group IV, claims 34-35, drawn to an agitation and heating apparatus.
Group V, claim 36, drawn to a method of testing for fluid delivery.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-V all relate to apparatus and methods suitable for use in chemical synthesis in a laboratory device. Each group has a different special technical feature corresponding to a different structure or method within the overall apparatus and method. Group I has as a special technical feature a mating tube and cavity coupling not required in groups II-V. Group II has as a special technical feature a holding reservoir not required in groups I, III-V. Group III has as a special technical feature a mathematically derived control scheme not required in groups I-II, IV-V. Group IV has as a special technical feature heating and agitation means not required for groups I-III, V. Group V has as a special technical feature the step of testing for a pressure change not required for groups I-IV.